RESPIRATORY AND ACID-BASE PHYSIOLOGY OF THE NEW ZEALAND ROCK LOBSTER, *JASUS EDWARDSII* (HUTTON).

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by
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'and perhaps you were never introduced to a lobster-' (Alice began to say 'I once tasted-' but checked herself hastily, and said 'No, never')

Alice's Adventures in Wonderland
Lewis Carroll, 1865

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CHAPTER 1

GENERAL INTRODUCTION

Although the study of respiration and acid-base physiology has traditionally centred on the vertebrates, there is now a growing number of studies which have concentrated on invertebrates. Among these, the very large number of physiological studies on the Class Crustacea perhaps reflects the remarkable diversity of habitats occupied by members of this group. Within the Order Decapoda, some species may live exclusively in water, including freshwater (e.g. *Austropotamobius pallipes*) or subtidal habitats (*Jasus edwardsii* and *Homarus*). Others are amphibious (e.g. the freshwater/land crab, *Holthuisana transversa*, and intertidal crabs such as *Carcinus maenas*), or fully terrestrial (e.g. *Gecarcinus* and *Cardisoma* spp.). Comparative physiological studies on members of the order Decapoda may therefore indicate the evolutionary changes enabling exploitation of new or diverse environments. Examination of the respiratory and acid-base changes of a particular species may also be prompted by features which are unique to that species, or to a particular group (e.g. the hermit crabs), a peculiarity of its response to changes in its environment (the emersion responses shown by *Austropotamobius* and *Carcinus* during environmental hypoxia), the changeable nature of its habitat (e.g. estuarine), or in some cases because it is a commercially exploited species (e.g. *Jasus*). In this last case, a study of the animals' ability to adapt or respond to various conditions will be of value in relation to its successful handling, manipulation, and maintenance by the industry.

Respiration may be divided into at least two levels. The first, internal respiration, describes oxygen usage and carbon dioxide production within the tissues. The second, external respiration, is

defined as the mechanisms involved in gas transfer between the animal and the medium. In order to respire efficiently an animal must possess means of moving the respiratory medium across the gas exchange surfaces, be able to take up oxygen from the medium, and then transport either oxygen to the tissues or the carbon dioxide produced to the gas exchange surface.

The major factor controlling ventilation in water-breathers is generally the availability of oxygen with little response to changes in CO_2 (Batterton and Cameron, 1978), whereas carbon dioxide usually provides the main ventilatory and circulatory drive in air-breathers. This difference relates to the different O_2 and CO_2 capacitances of the two media. The capacitance for CO_2 is similar in both water and air, and the capacitance coefficient for oxygen in air is also similar to that of CO_2. Water, however, carries only about one-thirtieth of the oxygen carried by air. Therefore, in aquatic animals ventilation is maintained at a high level in order to meet oxygen requirements. The high concentration of oxygen in air leads to ventilation rates that are rather lower than in water. Small variations in the concentration of carbon dioxide in either the haemolymph or the medium will produce a significant ventilatory response.

When an animal experiences a change in its environment or level of activity, resulting in a change in oxygen demand or the need to excrete carbon dioxide, it has several respiratory means at its disposal to counteract or lessen the impact of the perturbation. A change in the pattern or frequency of ventilation is a common response, resulting in a change in the rate of flow of air or water across the respiratory surfaces. Increased ventilation is often observed when an aquatic animal is subjected to hypoxia, an air-breather to hypercapnia, or in both groups as a result of the increased oxygen demands and accumulation of carbon dioxide during

exercise (McMahon, 1981; Taylor, 1982; Truchot, 1983). Modifications of gill perfusion may also facilitate oxygen uptake or carbon dioxide excretion by maximising the partial pressure gradients between the ambient medium and the respiratory circulation.

The regulation of acid-base state is usually described as the maintenance of pH (strictly, the hydrogen ion concentration) within limits around the 'set point' (Truchot, 1983). Rahn and Howell (1978), taking account of temperature effects on pH, more precisely described acid-base regulation as maintaining a constant relative alkalinity with respect to neutral water. The importance of regulating acid-base state results from the chemical nature of the hydrogen ion. Its high charge density means it can form strong hydrogen bonds with surrounding molecules. Thus changes in $[H^+]$ can change the structure and conformation of molecules, particularly proteins. Perturbations of the acid-base state may disrupt the integrity and functioning of membrane and enzyme systems. Moreover, since H^+ ions are an integral part of many biochemical reactions, local changes in the concentration of hydrogen ions may interfere directly with reaction rates in bio-chemical processes (Stewart, 1981).

The major causes of a change in the pH of the blood or intracellular space are changes in the partial pressure of carbon dioxide (PCO_2), a change in the total quantity of weak acid (A_{TOT}), or variations in the relative concentrations of strong cations and anions (the strong ion difference, SID; Stewart, 1978, 1981). Control of acid-base status is likewise effected through adjustments in any one of, or all, these variables. Acid-base regulation is also linked with a number of other physiological processes, including ion- and osmoregulation, respiration, circulation and nitrogen excretion (Truchot, 1983).

Animals vary in their tolerance to acid-base disturbances and

the precision with which they are able to counteract a displacement away from the set point. Those species which live in stable environments may be less inherently capable of compensating large variations in haemolymph pH compared to those which live in unstable environments. Thus, crustaceans living in tidal rock pools, where there may be large daily variations in oxygen content, temperature and salinity, might more readily correct, or at least tolerate, the resulting acid-base changes than those, such as *Jasus*, in whose habitat these factors remain more or less constant (Jouve-Duhamel and Truchot, 1983).

While ventilatory and/or circulatory modifications may act to maximise CO_2 elimination or O_2 uptake, conflicting requirements may change haemolymph acid-base status. For example, in hypoxic water ventilation is stimulated by the reduction in O_2. However, a higher $\dot{V}_w$ also promotes the excretion of CO_2, resulting in a respiratory alkalosis. Alternatively, an increase in ventilation or perfusion may be insufficient to alleviate the problems associated with O_2 supply and CO_2 elimination. When this occurs, acidification of the haemolymph may result directly from the accumulation of CO_2 or indirectly due to the build-up of anaerobic acid metabolites.

Respiratory and acid-base investigations have examined the response to a wide variety of internal and environmental perturbations. These include the effects of changes in temperature (e.g. McMahon and Burggren, 1981), variations in the oxygen (reviewed by Taylor, 1982 and McMahon, 1988) or carbon dioxide tension of the ambient medium (e.g. Cameron, 1978, 1981; Henry et al., 1981), emersion (e.g. Truchot 1975; deFur and McMahon, 1984 a, b), exercise (reviews by McMahon, 1981; Taylor, 1982; Cameron and Mangum, 1983), salinity (reviews by Cameron and Mangum, 1983; Truchot, 1983), the acid-base state of the water (Dejours and Armand, 1980), or combinations of different variables (e.g Taylor et al., 1977 a, b;

Dejours and Armand, 1983).

Although the response to a variety of perturbations have been examined in decapods, physiological studies investigating changes in respiration and acid-base regulation have usually been restricted to a few common species, with little known of other groups. The cardiorespiratory physiology of crabs, particularly *Carcinus*, *Cancer* and *Callinectes* have been the most intensively studied. True lobsters and crayfish, while receiving less attention in this respect than the crabs, are also fairly well understood. In contrast, the palinurids or spiny lobsters, which superficially resemble the clawed lobsters, have been largely ignored in respiratory investigations. Belman (1975) described some physiological features of the circulation in *Panulirus interruptus* and Vermeer (1987) recorded changes in some haemolymph acid-base variables during air exposure of *Panulirus argus*. Changes in heart activity and blood pressures in *Panulirus japonicus* have recently been studied by Kuramoto (1990).

The morphological characteristics of the crustacean cardio-respiratory system differ from those of other animal classes in a number of ways. Crustaceans are stated to have an 'open' circulation, although for decapods, which have a well-organised arterial system, this term can be somewhat misleading (McMahon and Wilkens, 1983; Taylor, 1982). Paterson (1968) described the venous system of *Jasus lalandii* as a series of interconnecting sinuses. While this suggests little control over the venous circulation, McMahon and Burnett (1990) stated that there was better control of pressure and flow than had previously been thought. Circulation is powered by a single-chambered heart, blood entering the heart through ostia and exiting via arterial vessels. Unlike vertebrates, the respiratory pigment, haemocyanin, is not located within discrete cells, but is dissolved within the haemolymph.

The gill systems of crustaceans are also simpler than those seen

in the lower vertebrates, although they perform the same functions of respiration and ionoregulation. Two distinct gill types are found in the Decapoda. The phyllobranchiate, or lamellate, gills of most brachyuran crabs are somewhat similar in structure to those of fish. Filamentous gills occur as a simple (trichobranchiate) or branched (dendrobranchiate) filament, the former in the macrurans and the latter in some forms of shrimp (McMahon and Wilkens, 1983). The flow of water across the gills is countercurrent in both trichobranchiate and lamellate gills (McMahon and Wilkens, 1983; Rogers, 1982). However, Rogers also pointed out that, because of the direction of haemolymph flow within the gill filament, countercurrent exchange will be less effective than in fish and in those crustaceans with lamellate gills. The circular cross-section of the trichobranchiate gills of macrurans may make them more resistant to collapse than the lamellate gills of brachyurans, although in some crabs inhabiting the littoral zone strengthening of the gills may prevent their coalescence in air (Taylor and Butler, 1978).

All but the most primitive Crustacea have some means of moving the respiratory current across the gas exchange surface. In decapods, ventilation is achieved by beating of the scaphognathites which, through the development of a sub-ambient pressures within the branchial chambers, draws water across the gill surfaces.

The arrangement of the gills within the branchial chamber in *Jasus edwardsii* is similar to those described by Rogers (1982) for *J. novaehollandiae* and for *J. lalandii* by Paterson (1968) (Fig. 1.1). All have 21 gills on each side arranged in a 2/3/3/4/4/4/1 (anterior to posterior) format. The length of individual gills varies to fit within the shape of the branchial cavity, but each tapers at the tip, with the distal filaments shorter than those at the base. The flow of water is generated by the beating of the scaphognathite which lies within the pre-branchial chamber. Water is drawn in across the gills

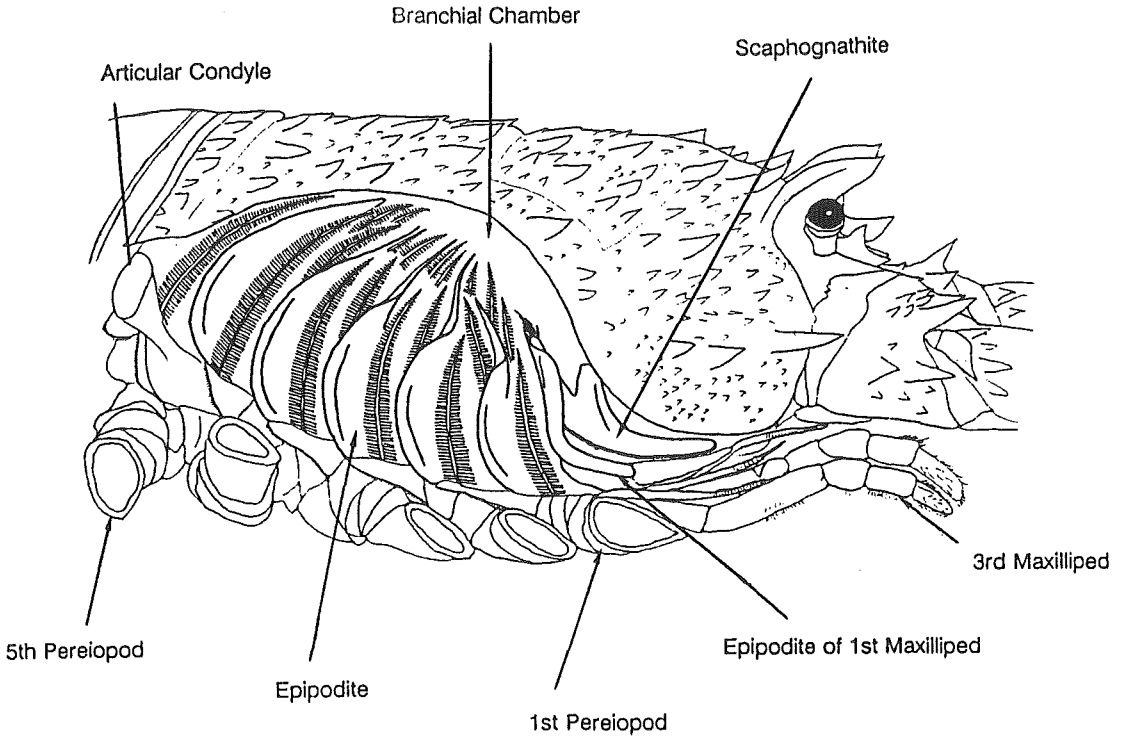

Fig. 1.1 Line drawing showing the position of the scaphognathite and arrangement of the gills in *Jasus edwardsii*. The epipodite of the second maxilliped and two anterior gills are not shown.

at the base of the walking legs, before passing anteriorly through the pre-branchial chamber and out via the exhalent aperture. The diameter of the pre-branchial chamber is not necessarily fixed, since the floor is formed by the epipodite of the first maxilliped. When moved, this can either widen or narrow the pre-branchial chamber. Neither are the gills 'static' organs, since movement of the legs can change the position of the podobranch relative to the other gills.

Within the Order Decapoda, there are two major body-types: crab-like forms which includes both brachyurans and anomurans and macrurous forms which include lobsters, spiny lobsters, shrimps and crayfish (the macrurans). Several features of the anatomy of the Macrura distinguish them from the crabs. The most obvious feature, as implied by their name, is the retention of a large, abdominal muscle mass (the tail). This has been reduced to little more than an egg-carrying organ in the crabs. The thorax is also generally longer in the crayfish and lobsters than in the crabs. A significant feature in relation to respiration is that most macrurans possess filamentous gills (either trichobranchiate or dendrobranchiate) whereas the gills of brachyurans are plate-like (phyllobranchiate).

Two large, marine macrurans of the family Palinuridae are found in New Zealand waters: the packhorse lobster, *Jasus verreaux* and the New Zealand crayfish or red rock lobster, *Jasus edwardsii* (Hutton). By far the more widespread is *J. edwardsii*. It ranges from the far north to areas in the south below Stewart Island, and half way up the west coast of the South Island (Anonymous, 1980).

The preferred habitat of *J. edwardsii* is rocky bottom in relatively deep water (approximately 45m), where it can find shelter from its abundant predators. It does occur in shallower, more sandy areas, although this is usually limited to inshore migrations (Street, 1969, Anonymous, 1980). *Jasus* is known for its long migrations, which are often associated with moulting or reproductive

cycles (Street, 1969). More common movements are those associated with feeding, although they are also known to perform tail flips, usually in response to the presence of a predator. This 'escape response' is exhibited by many macrurans, and involves a forceful flexion, or 'flick', of the powerful abdominal muscles of the tail.

Jasus edwardsii is perhaps the most important crustacean in the New Zealand fishing industry. However, the majority of studies have concentrated on its ecology, and little is known of its physiology. Binns and Peterson (1969) detailed the use of the antennal gland in nitrogen excretion, and Ellerton et al. (1977) studied its haemocyanin molecule, but there are few other studies of the physiology of this species. Thus, investigation of the physiology of *Jasus* provides much-needed information on a group of animals neglected in crustacean respiratory and acid-base studies. Additionally, with the development of a 'live export' industry, accurate knowledge of its response to environmental perturbations would be invaluable in ensuring higher survivorship and quality of the exported animals. The emphasis in this examination of the physiology of *J. edwardsii* is on the integrated response of the respiratory and acid-base systems to lessen the impact of perturbations in either internal or external conditions.

Included in this study are measurements of respiratory and acid-base variables during settlement, hypoxia, air exposure, exercise, and exercise coupled with emersion. In addition, some acid-base indicators were also obtained from animals destined for live export. Each chapter draws on information provided by those preceding, with the view to determining the limits of control and regulation achieved by *Jasus* under conditions it may experience during capture and handling.

ABBREVIATIONS

The symbols used throughout this thesis follow the nomenclature of Dejours (1981). Some commonly used symbols and abbreviations are given below.

Abbreviation	Description	Units
$\dot{M}O_2$	oxygen consumption	$\mu mol.kg^{-1}.min^{-1}$
$\dot{V}_w$	minute ventilation volume	$ml.kg^{-1}.min^{-1}$
$\dot{V}_w/\dot{M}O_2$	convection requirement	$ml.\mu mol^{-1}$
f_{sc}	respiratory frequency	$beats.min^{-1}$
f_H	heart frequency	$beats.min^{-1}$
PO_2	partial pressure of oxygen	Torr
PCO_2	partial pressure of carbon dioxide	Torr
CO_2	concentration of oxygen	$\mu mol.l^{-1}$
$[HCO_3^- + CO_3^{2-}]$	bicarbonate concentration	$meq.l^{-1}$
H^+_m	metabolic acid	$meq.l^{-1}$
$[NH_3]$	ammonia concentration	$mmol.l^{-1}$
I	inspired, e.g. P_IO_2	
E	expired, e.g. P_EO_2	

CHAPTER 2

VENTILATORY RESPONSE TO EXPERIMENTAL ACCLIMATION AND HYPOXIA

ABSTRACT

Changes in ventilation were measured during recovery from handling stress and operative procedures (acclimation) and during progressive hypoxia in a closed volume of sea water at 15°C. High rates of oxygen uptake ($\dot{M}O_2$ = 25 μmol.kg^{-1}.min^{-1}), ventilation volume ($\dot{V}_w$ = 542 ml.kg^{-1}.min^{-1}) and total scaphognathite frequency (f_{sc} = 324 bpm) were recorded at the start of settlement, while oxygen extraction efficiency (%Ext) was relatively low at around 20%. $\dot{M}O_2$ and $\dot{V}_w$ decreased most rapidly during the first 8 hours of acclimation, while the rate of decrease of f_{sc} was almost linear up to 24h. All three variables continued to decrease up to 48h post-handling. There was little change in %Ext over the 48h, indicating that oxygen uptake varied mainly with ventilation. At least 48 hours was required before settled levels of ventilatory variables were attained.

Resting metabolism was low in *Jasus* compared to other crustaceans, $\dot{M}O_2$ measuring only about 10 μmol.kg^{-1}.min^{-1}. Ventilation volume, and hence the convection requirement, $\dot{V}_w/\dot{M}O_2$, was relatively high suggesting inefficiency of the gas exchange mechanisms in this species. $\dot{M}O_2$ was maintained down to 78 Torr ($PO_{2(crit)}$) after which it fell with decreasing P_IO_2. Both ventilation volume and frequency increased with deepening hypoxia, both becoming significantly higher than normoxic values at around 90 Torr. Hypoxia also resulted in a reduction in extraction efficiency, %Ext decreasing from 31% in normoxia to a constant 13% below 78 Torr. The relationships between

%Ext, $\dot{V}_w$ and $\dot{M}O_2$ indicate that above $PO_{2(crit)}$ ventilation was increasing at a rate faster than that required for adequate delivery of oxygen and is supported by the calculated increase in the amount of oxygen made available to the gills down to P_IO_2 = 78 Torr. Below $PO_{2(crit)}$ ventilation increased more slowly than the oxygen content of the water decreased, suggesting that ventilation was approaching its upper limit. Recovery from hypoxia produced a steady increase in %Ext and a transient increase in $\dot{M}O_2$ above the pre-hypoxic level, while ventilation volume and frequency decreased. The amount of oxygen used during recovery was greater than the O_2 'lost' during hypoxia. This suggests that the oxygen debt consisted not only of repayment of the decrease in $\dot{M}O_2$ below resting levels (the O_2 deficit), but also of the additional energy required to power the elevated pumping frequencies during hypoxia and possible remetabolism of lactate.

INTRODUCTION

While the capacitances of air and water are similar for carbon dioxide, for oxygen it is much lower in water than it is in air. At any given PO_2, water carries only around one thirtieth of the oxygen available in air. As a consequence, aquatic animals generally vary ventilation in relation to the ambient oxygen tension.

In the literature animals are often classified as either oxygen 'conformers' or 'regulators'. These terms appear analogous to those used in osmoregulation studies, but are misleading since two different kinds of variables are being compared (a rate and a concentration). More recent studies have described an animal's oxygen uptake either as varying with the external oxygen tension (oxygen-dependent) or as being maintained at a constant level despite a change in PO_2 (oxygen-independent). These two terms refer to animals

at either end of the spectrum; more usually, an animal may exhibit respiratory independence over part of the PO_2 range while it is dependent over the remainder (Herreid, 1980).

Many studies have characterised the respiratory response to a decrease in the availability of oxygen in crustaceans, and have examined the dependence of oxygen uptake on the ambient oxygen tension, and the critical PO_2 below which the resting level of oxygen uptake is no longer maintained ($PO_{2(crit)}$). Other factors, such as salinity and temperature may influence the degree of respiratory independence. In *Carcinus maenas* increasing the temperature raises the critical PO_2 while reducing salinity lowers it (Taylor, Butler and Al-Wassia, 1977b). The experimental procedures used may also change a species from being apparently oxygen-dependent to oxygen-independent. Thomas (1954) used fettered lobsters, *Homarus vulgaris*, when he examined their response to declining oxygen tensions and concluded that their oxygen uptake fell with a decrease in PO_2. Using unfettered lobsters Spoek (1974) discovered that *Homarus* was able to maintain its oxygen uptake down to about 20% saturation. Innes (1985) also showed that the degree of respiratory independence in the mantis shrimp, *Heterosquilla tricarinata*, was much greater in inactive animals. In these studies it seems that the initial metabolic rate determined whether the animal was able to maintain its oxygen uptake at the normoxic level or whether it fell with decreasing PO_2.

This study presents results on the changes in ventilatory variables occurring in the rock lobster, *Jasus edwardsii*, during recovery from handling stresses and operative procedures, and of the effects of short-term, progressive hypoxia on respiration following 48h acclimation to the experimental chamber.

MATERIALS AND METHODS

Rock lobsters, *Jasus edwardsii*, of either sex and weighing between 300 and 730 g were used in these experiments. All animals had hardened shells and were judged to be in intermoult. The animals were held in a recirculating, filtered seawater system (temperature = 17 ± 2°C; salinity ~36‰) and were fed regularly with mussels. They were deprived of food for at least a week before experimentation.

Preliminary experiments (presented below) were performed to determine the amount of time required after disturbance and handling for stable values of ventilatory variables to be reached. Oxygen uptake, inspired and expired $P\mathrm{O}_2$ and ventilation frequency were measured on 5 - 6 animals using the methods described below. On the basis of these results the animals were put into the respirometer at least 48h before hypoxia experiments were conducted.

Experimental Protocol

The rate of oxygen consumption during hypoxia was measured on 11 rock lobsters using a closed-box technique. Animals were put into a Perspex chamber (approximate volume = 4.4L) which could be closed from its normal flow-through mode by a series of three-way taps. The respirometer was immersed in a thermostatted water bath at 15 ± 1°C and covered with black plastic sheeting to prevent visual disturbance of the animals. Circulation within the chamber was maintained using magnetic stirrer bars located at each end of the respirometer and separated from the animal by perforated plastic partitions.

During the initial 48h acclimation period normoxic water was pumped through the chamber, and measurements of settled respiratory variables were made. The respirometer was then closed and oxygen consumption measured as the oxygen tension within the chamber decreased from ~140-150 Torr down to about 30 Torr. Estimates of the

change in PCO_2 based on the measured change in PO_2 and RQ = 0.8, indicated a rise of about 3 Torr during each trial. Such a small change in PCO_2 has been found to have no influence on ventilation in aquatic crustaceans (Batterton and Cameron, 1978; O'Mahoney and Full, 1984).

The time course of each hypoxic experiment was dependent on the size of the animal relative to the respirometer volume and on individual rates of oxygen uptake, but generally varied between 2-4h. Following hypoxia the respirometer was opened and flushed with normoxic sea water. On 5 animals further measurements were made over the 6h following hypoxia.

Oxygen Consumption

Partial pressures were measured by two methods. In the first discrete samples (approximately 5 ml) were removed from the chamber and the volume replaced with fresh sea water (a change of about 0.1% of the total volume of the respirometer). The PO_2 was then measured with a PO_2 electrode (Strathkelvin 1302) thermostatted to 15 ± 1°C connected to an oxygen meter (Strathkelvin 781b). Alternatively, during hypoxia only, water was continuously recirculated past a thermostatted external Beckman O_2 electrode (Beckman 13992) connected to a chart recorder (Beckman SE120). Both electrodes gave essentially similar results, so either was used to measure PO_2 changes once the respirometer was closed.

Two methods were used to calculate oxygen uptake. Water flowed through the respirometer in the control period before the hypoxia experiments and in the period following hypoxia. Oxygen consumption was then calculated on the basis of the drop in PO_2 across the respirometer ($PO_{2inflow}$ - $PO_{2outflow}$) and on the water flow rate ($\dot{V}_{H2O}$, $L.min^{-1}$) according to the equation:

(Equation 2.1):

$$\dot{M}O_2 = \frac{\dot{V}_{H2O} \times \alpha O_2 \times \Delta PO_2}{\text{weight}} \quad (\mu\text{mol.kg}^{-1}.\text{min}^{-1}),$$

where αO_2 is the solubility coefficient of oxygen in water in μmol.L^{-1}.$Torr^{-1}$ (Dejours, 1981).

During the hypoxic run oxygen uptake was calculated on the decrease in inspired oxygen tension, P_IO_2, and the respirometer volume ($V_{(respirometer)}$ over a known time interval:

(Equation 2.2):

$$\dot{M}O_2 = \frac{V_{(respirometer)} \times \alpha O_2 \times \Delta P_IO_2}{\text{time} \times \text{weight}} \quad (\mu\text{mol.kg}^{-1}.\text{min}^{-1})$$

Control respirometers, without animals, were run to test for oxygen uptake by micro-organisms (usually 5-10% of the change measured when an animal was in the respirometer). Adjustments were made to correct for any change in PO_2.

Oxygen Extraction

Five animals were fitted with a rubber mask for measurements of the PO_2 of the mixed expired water (P_EO_2). The mask was fitted across the mouth and sealed with a cyanoacrylate glue around the edges of the carapace to separate the expired water from the inhalent current. Movements of the mouthparts, limbs and antennules were not restricted when the mask was in place. Testing for leaks was achieved by injecting a small amount of red dye at the base of the limbs and noting the flow through the mask. A canula was inserted into the projecting tube of the mask for collection of the mixed expired water. Samples were slowly withdrawn and PO_2 measured using the Strathkelvin PO_2 electrode.

Ventilation Frequency

To minimise handling stresses and the time exposed to air, animals used for the determination of total respiratory frequency (f_{sc} = the sum of the left and right scaphognathites) were subjected only to the operative procedures involved in obtaining scaphognathite recordings and not to the attachment of a mask or water sampling cannulae. It is possible that the two treatments may have had different effects on ventilatory or respiratory variables but the quantitative effect is unknown. It took somewhat longer to implant electrodes (about 30 - 40 min) than it did to attach the respiratory masks or cannulae (approx. 10 min.). Nevertheless, in both groups handling and exposure to air were involved prior to the experiments.

Recordings of ventilation frequency during experimental acclimation and hypoxia were made using an impedance technique similar to those used for *Cancer magister* by McDonald et al. (1977) and for *Orconectes rusticus* by Wilkes and McMahon (1982). Fine silver wires, bared for ~4mm at the tip, were inserted through small holes drilled through the carapace on either side of the scaphognathites and sealed in place with hot glue. Repeatable positioning of the electrodes with respect to the scaphognathites could be made by the common patterning of spines in *Jasus edwardsii*. The electrodes were soldered to a fine lead which passed through a rubber bung positioned in the lid of the chamber. The impedance signal was detected using Strathkelvin Impedance Couplers (Biosciences A100 Power Supply) and then amplified and recorded on a Gould 2200s or 2202 oscillographic recorder. The reliability of the impedance method in determining respiratory frequency has been established by simultaneous pressure and impedance recordings (McDonald et al., 1977).

Calculations

(Equation 2.3):

percentage oxygen extraction

$$\%Ext = \frac{(P_IO_2 - P_EO_2)}{P_IO_2} \times 100$$

(Equation 2.4):

ventilation volume ($ml.kg^{-1}.min^{-1}$)

$$\dot{V}_W = \frac{\dot{M}O_2}{\alpha O_2 \times \Delta PO_2}$$

(Equation 2.5):

convection requirement ($ml.\mu mol^{-1}$)

$$\dot{V}_W/\dot{M}O_2$$

(Equation 2.6):

C_IO_2 ($\mu mol.L^{-1}$)

$$P_IO_2 \times \alpha O_2$$

(Equation 2.7):

concentration of oxygen delivered to the gills ($\mu mol\ O_2.kg^{-1}.min^{-1}$)

$$\frac{C_IO_2 \times \dot{V}_W}{1000}$$

The symbols used in the above equations are described at the end of Chapter 1.

Statistical Treatment

The data was initially compared with an ANOVA. Testing for differences between means was made using Fisher's Least Significance Difference Significance was designated at $p<0.05$. All data are given as the mean ± 1 SEM unless otherwise stated.

RESULTS

Experimental acclimation

During the course of these and other experiments *Jasus* was permitted 48h to recover from the effects of handling stresses, air exposure, operative procedures and to acclimate to the experimental apparatus. Establishment of the time course of recovery from these stresses was essential to ensure resting levels of the physiological variables measured in subsequent experiments.

Figs 2.1 - 2.5 illustrate the changes in a number of respiratory variables over the 48h acclimation period. Oxygen consumption was initially high, measuring a mean 24.6 ± 2.7 $\mu mol.kg^{-1}.min^{-1}$ (Fig. 2.1). After 8h it had fallen significantly to half this value ($p<0.02$) and continued to decrease by a further 25% to 9.4 ± 1.4 $\mu mol.kg^{-1}.min^{-1}$ up to 48h post-handling.

Minute ventilation volume ($\dot{V}_w$) was calculated from the $\dot{M}O_2$ data and from the difference between inspired and expired PO_2, and showed a similar time course to that of $\dot{M}O_2$ during acclimation (Fig. 2.2). From an initial mean pumping rate of 542 ± 58 $ml.kg^{-1}.min^{-1}$ ventilation decreased to less than a third of this value by 48h. Again, the major portion of restoration to the settled ventilation volume occurred within the first 8h of acclimation, since $\dot{V}_w$ at that time was still twice the final value reached at 48h ($p<0.05$).

Percentage extraction of oxygen from the inspired water (%Ext) increased slightly with acclimation (Fig. 2.3). It rose from 22.2 ± 2.3% at 1h to 29.2 ± 3.3% after 48h (significantly different from the 48h value at 4 and 8h, $p<0.05$). Most of the change recorded in %Ext occurred between 24 and 48h, as there was little change in the mean values up to 24h (value at 24h = 23.7 ± 2.3%). The values reached at the end of the acclimation period were somewhat variable, and in one animal more than 50% of the available oxygen was extracted from the

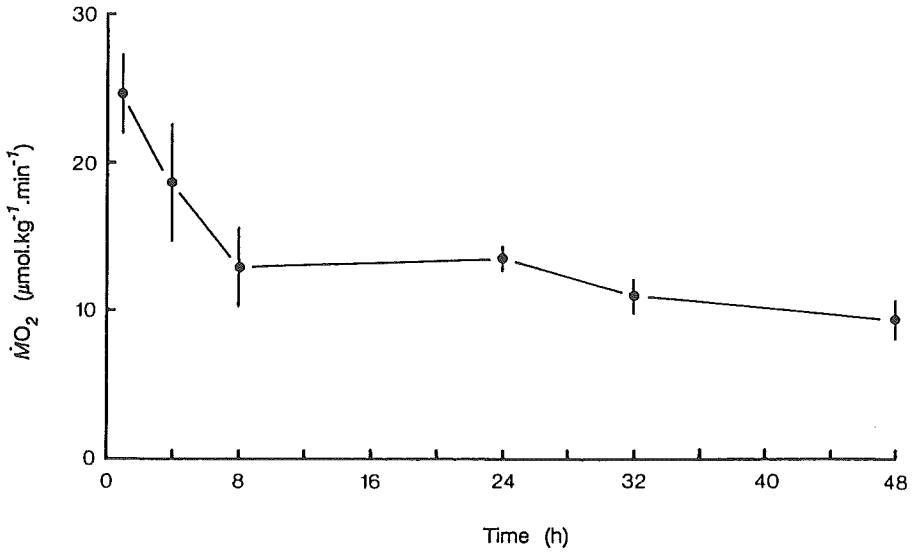

Fig. 2.1 Changes in oxygen consumption ($\dot{M}O_2$) during settlement at 15°C. 0h = return of the animals to water following handling or electrode implantation in air. Data are given as mean ± 1 SEM. n = 5.

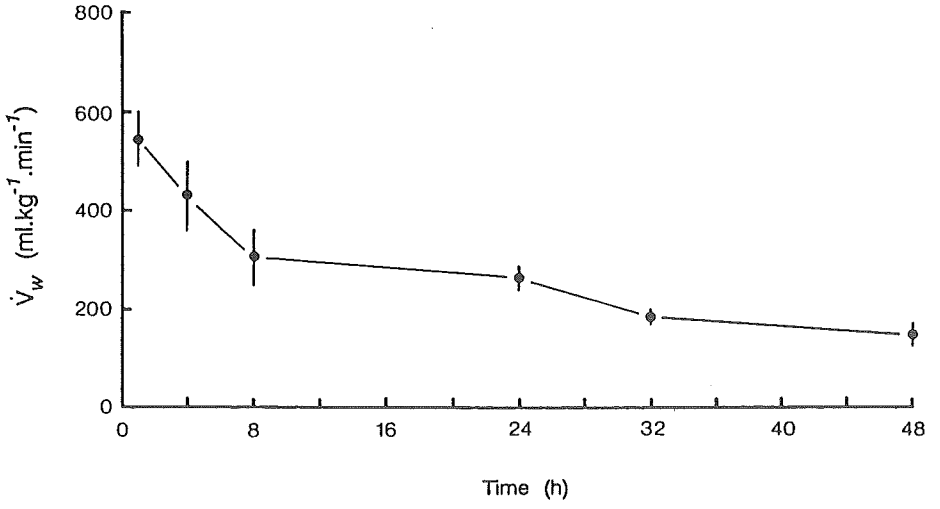

Fig. 2.2 The effect of recovery from handling stress on minute ventilation volume ($\dot{V}_w$) in *Jasus edwardsii* at 15°C. Data = mean ± 1 SEM, n = 5.

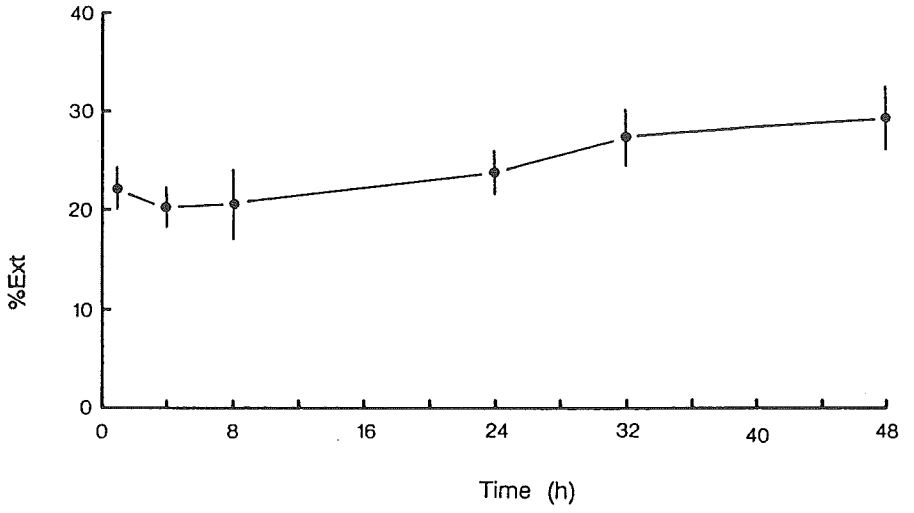

Fig. 2.3 Changes in percentage extraction of oxygen from the inspired water (%Ext) during settlement. Temperature = 15°C. Data are presented as mean ± 1 SEM, n = 5.

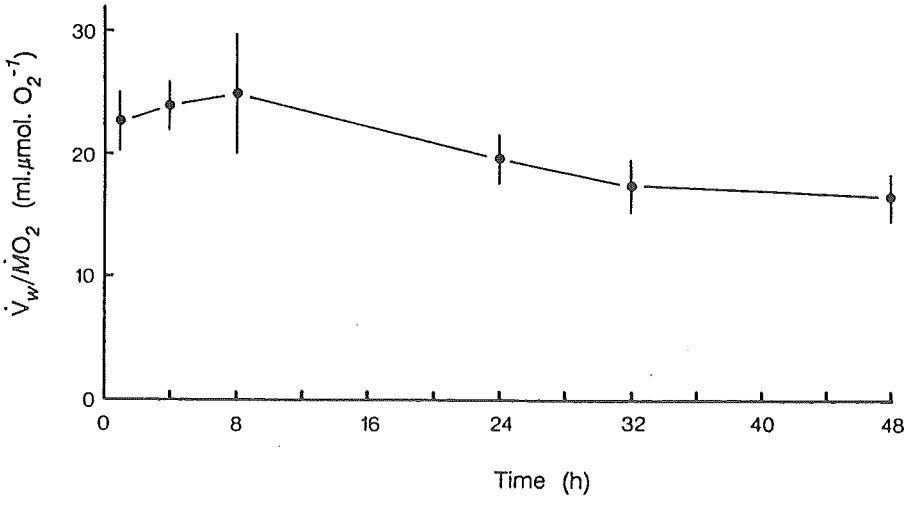

Fig. 2.4 Changes in the convection requirement, $\dot{V}_w/\dot{M}O_2$, during settlement at 15°C. Data = mean ± 1 SEM, n = 5.

inspired water.

The ventilatory requirement ($\dot{V}_w/\dot{M}O_2$) is shown in Fig. 2.4. The value at 8h was significantly higher than the value at 48h (25 ml.μmol O_2 at 8h, 16.4 ml.μmol O_2 at 48h, $p<0.05$). None of the other values was significantly different to that at 48h.

Total ventilation frequency (f_{sc}, Fig. 2.5) fell during acclimation, although it decreased rather more linearly over the first 24h than did $\dot{V}_w$ or $\dot{M}O_2$. As for these latter variables, f_{sc} continued to fall even between 24 and 48h, although at a rate 5 times slower than in the previous 24h.

Hypoxia

Oxygen consumption

The rate of oxygen consumption during progressive 'self-induced' hypoxia is shown in Fig. 2.6. In normoxic water (~140-150 Torr), mean $\dot{M}O_2$ was 10.4 ± 1.6 μmol.kg^{-1}.min^{-1}, which was not significantly different to that recorded at the end of the acclimation period. Oxygen consumption was maintained at approximately this level down to a P_IO_2 of 80-100 Torr. Below 60-80 Torr there was an almost linear decrease in $\dot{M}O_2$ with decreasing P_IO_2, linear regression through the mean values yielding an r^2 value of 0.999. Although this rate change occurred at an apparent critical PO_2 (P_{crit}) of around 78 Torr, $\dot{M}O_2$ became significantly different from resting $\dot{M}O_2$ at 40 Torr ($p<0.05$).

Extraction efficiency

Simultaneous measurements of inspired and expired water PO_2 permit the calculation of the proportion of the dissolved oxygen extracted at the different levels of oxygenation. These results are presented graphically as the percentage extraction, %Ext (Fig. 2.7). As the level of hypoxia deepened, extraction efficiency generally decreased. At rest, %Ext was 31 ± 4%, but at a mean inspired tension

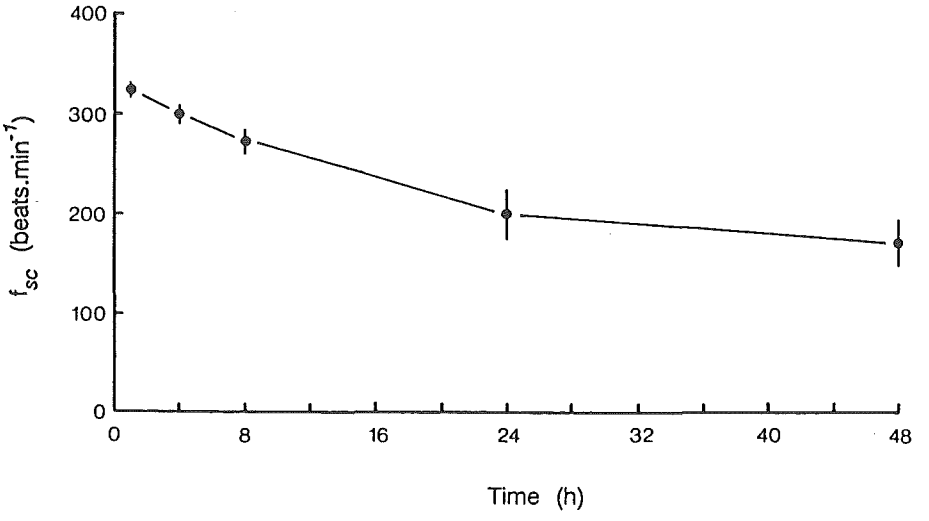

Fig. 2.5 Changes in f_{sc} (total rate = sum of the left and right frequencies) during settlement in *Jasus* at 15°C. Data are given as mean ± 1 SEM. n = 6.

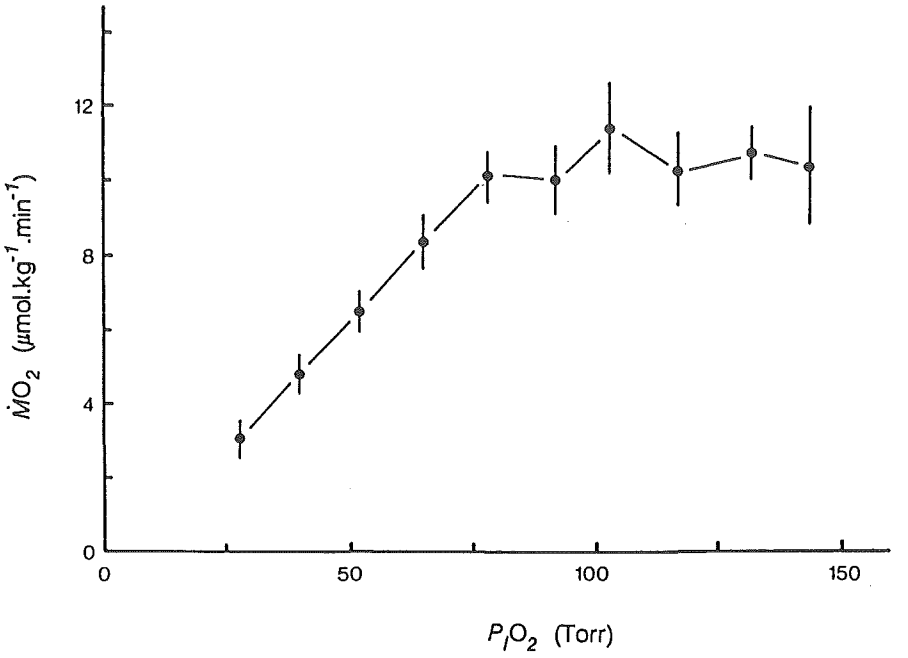

Fig. 2.6 Changes in $\dot{M}O_2$ during progressive hypoxia (P_IO_2 = 144 - 30 mmHg). All data are given as mean ± 1 SEM. n = 11, except at 144 and 30 mmHg, where n = 5.

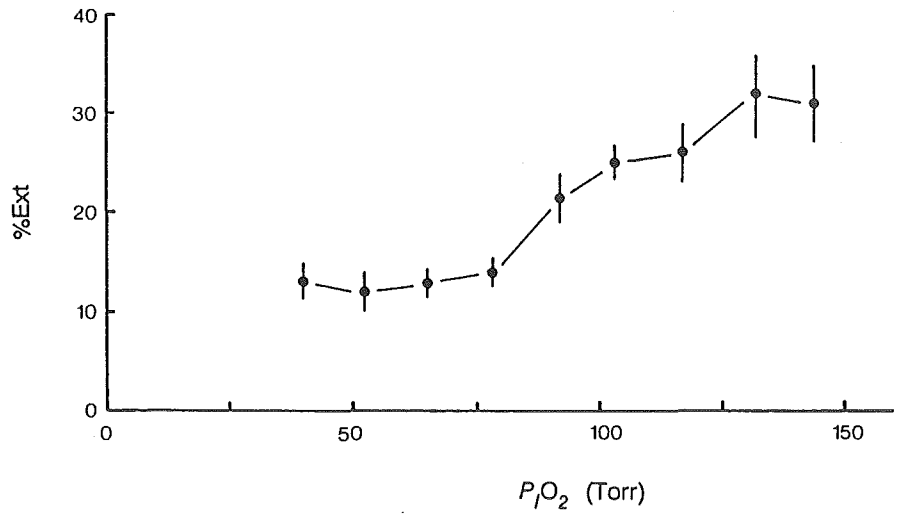

Fig. 2.7 The effect of progressive hypoxia on oxygen extraction efficiency (%Ext) in *J. edwardsii* at 15°C. Data are given for n = 5 animals ± 1 SEM.

of 92 Torr extraction had significantly fallen to only 22% removal of the available oxygen ($p<0.02$). At values below 78 Torr, percent extraction remained relatively constant at around 13%, which was less than half the percentage extraction of oxygen during normoxia.

Ventilation Volume, $\dot{V}_w$

Minute ventilation volumes calculated for 5 animals are illustrated in Fig. 2.8. In settled lobsters mean $\dot{V}_w$ was 159 ± 38 $ml.kg^{-1}.min^{-1}$. Branchial ventilation rose with decreasing PO_2, although in the initial stages it climbed only slowly. At 78 Torr $\dot{V}_w$ was 365 ± 32 $ml.kg^{-1}.min^{-1}$ which was significantly higher than $\dot{V}_w$ at 144 Torr ($p<0.001$). The most rapid rate of increase occurred between 92 and 78 Torr, $\dot{V}_w$ at the latter PO_2 measuring about 610 $ml.kg^{-1}.min^{-1}$. Ventilation continued to increase with progressively lower inspired oxygen tensions but below 78 Torr the rate of increase was less than at higher oxygen tensions. At 40 Torr $\dot{V}_w$ reached a maximum of 752 ± 55 $ml.kg^{-1}.min^{-1}$, which was about 4.5 times higher than the settled level.

The ventilatory requirement ($\dot{V}_w/\dot{M}O_2$) showed a strong dependence on the level of oxygenation (Fig. 2.9). At rest, $\dot{V}_w/\dot{M}O_2$ was 14.8 $ml.\mu mol^{-1}$. As P_IO_2 decreased the ventilatory requirement increased, albeit slowly at first, and was significantly different from the normoxic value at 92 Torr ($p<0.05$). The main deviation point was also around this inspired oxygen tension, where a considerably higher rate of increase was observed. At the lowest tension measured, 40 Torr, the ratio of ventilation to oxygen uptake had risen 10-fold over the level at 144 Torr.

The amount of oxygen made available at the gills was calculated using ventilation rates and the concentration of oxygen in the inspired water (Fig. 2.10). Oxygen delivery increased down to an inspired tension of 78 Torr (statistically significant, $p<0.001$).

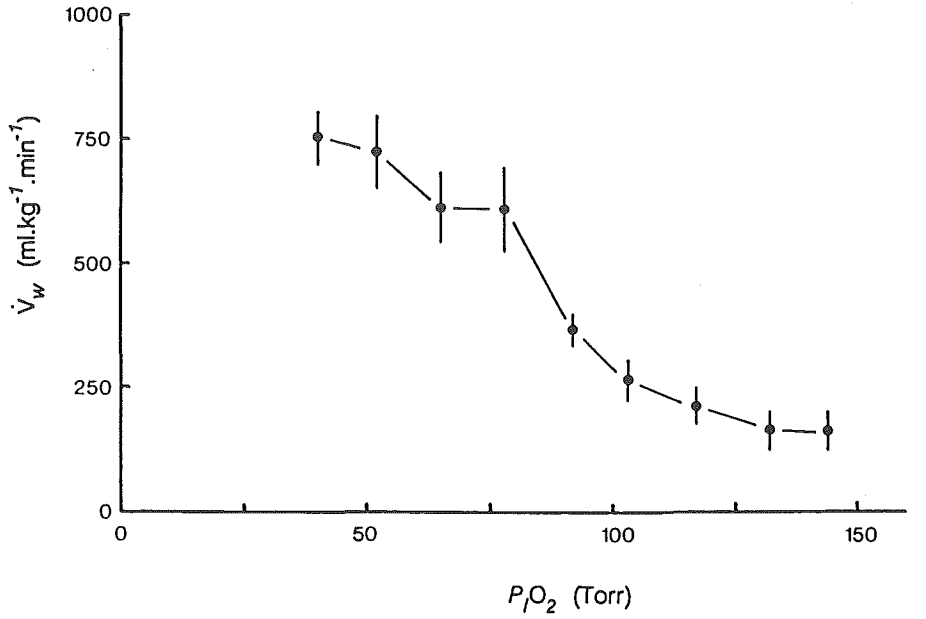

Fig. 2.8 Changes in ventilation volume ($\dot{V}_w$) during exposure to progressive hypoxia at 15°C. Data given = mean ± 1 SEM, n = 5.

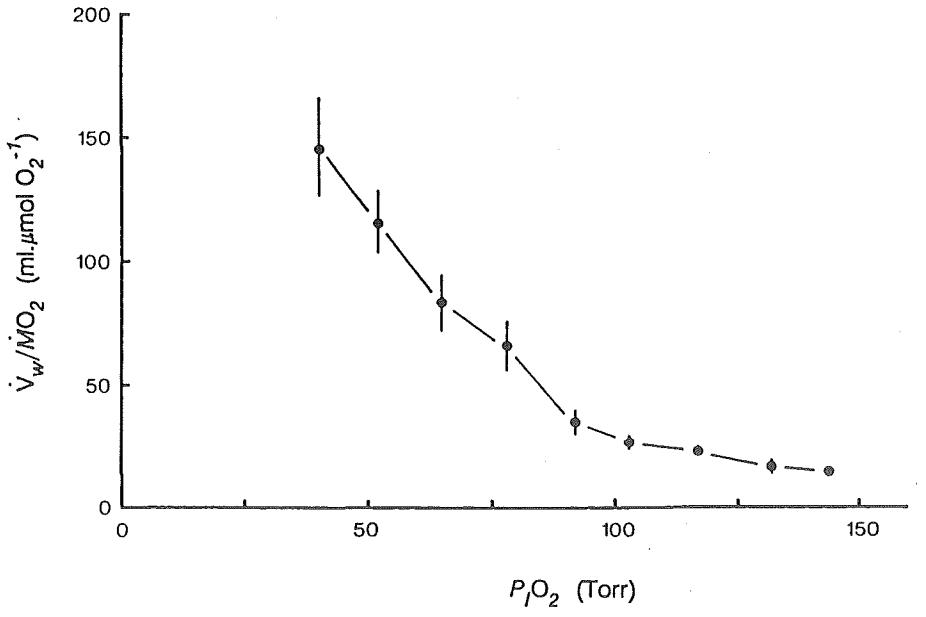

Fig. 2.9 Changes in the convection requirement ($\dot{V}_w/\dot{M}O_2$) during hypoxia. Data are given as mean ± 1 SEM for n = 5 animals. Where no error bars are shown they lie within the symbol.

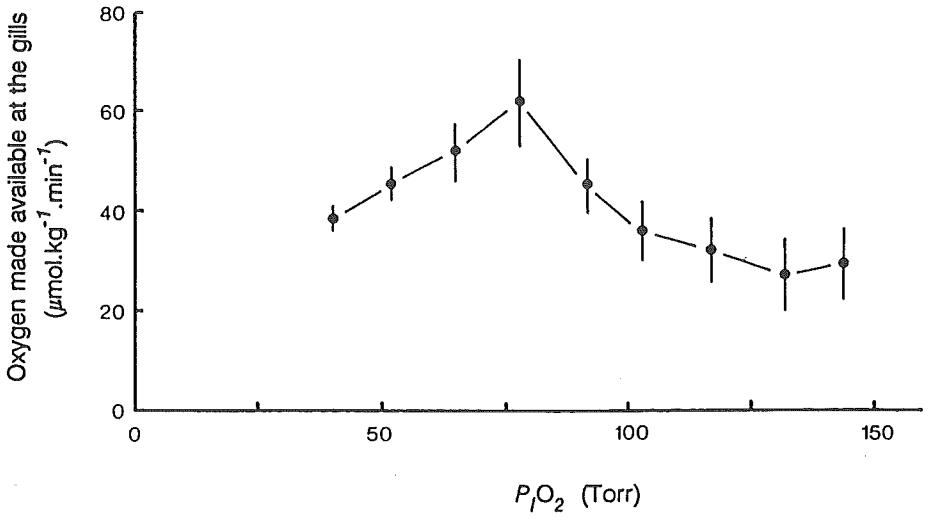

Fig. 2.10 Changes in the amount of oxygen made available at the gills (calculated as $\dot{V}_w.C_IO_2 \div 1000$) during exposure to declining oxygen tensions at 15°C. Means ± 1 SEM, n = 5.

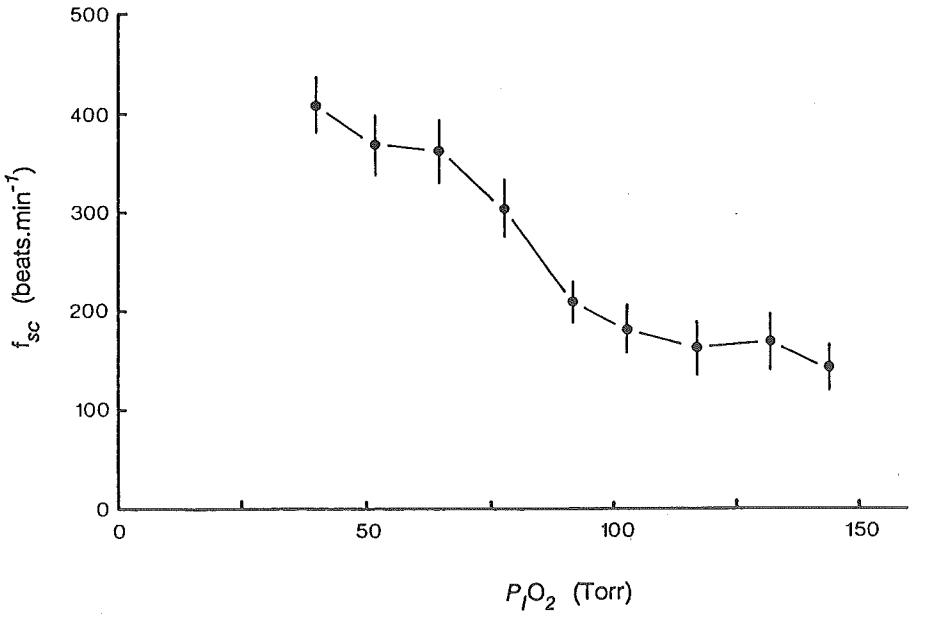

Fig. 2.11 The effect of progressive hypoxia (P_IO_2 = 144 - 30 mmHg) on total f_{sc} at 15°C. Data = mean ± 1 SEM, n = 4.

Below this level steadily less oxygen was made available but at 65 Torr it was still significantly greater than the amount at 144 Torr $p<0.02$).

Ventilation Frequency

Changes in total ventilation frequency (f_{sc}) of 4 animals during progressive hypoxia are shown in Fig. 2.11. In normoxia the frequency of ventilation was 144 ± 23 bpm. There was only a slight increase as P_IO_2 decreased to about 100 Torr, but the value at 103 Torr, 183 bpm, was significantly higher than at 144 Torr ($p<0.01$). The greatest rate of increase occurred between 92 and 78 Torr, at the latter PO_2 f_{sc} measuring 306 ± 31 bpm. It continued to increase over the duration of the experiments, and reached a final value of 409 ± 30 bpm, constituting a three-fold increase in respiratory frequency.

Recovery from Hypoxia

The changes during recovery from hypoxia are illustrated in Figs. 2.12 - 2.16. After 1h oxygen uptake had increased to more than double the pre-hypoxic rate, rising to about 23 $\mu mol.kg^{-1}.min^{-1}$ (Fig. 2.12). With further recovery it decreased and was not significantly different from the resting level by 3h.

Percent extraction increased steadily throughout recovery, although the change was not particularly rapid (Fig. 2.13). After 4h %Ext was still significantly below the pre-treatment extraction efficiency ($p<0.02$), measuring only about 70% of the original value.

Both ventilation volume ($\dot{V}_w$, Fig. 2.14) and frequency (f_{sc}, Fig. 2.15) decreased from the peaks recorded at ~40 Torr. The means of both variables were not significantly different from their resting values by 2h and 3h, respectively. Restoring normoxic water also meant ventilation became more efficient in providing oxygen to the

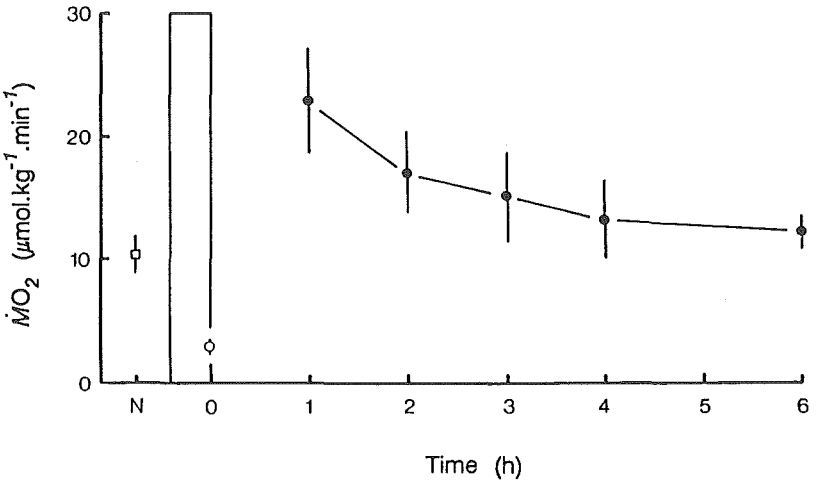

Fig. 2.12 $\dot{M}O_2$ at rest in normoxia (N,□), at the end of hypoxia (0h,○) and during recovery in normoxic water (●). Vertical bar represents the period of hypoxic exposure (between 2 - 4h). Means ± 1 SEM, n = 5.

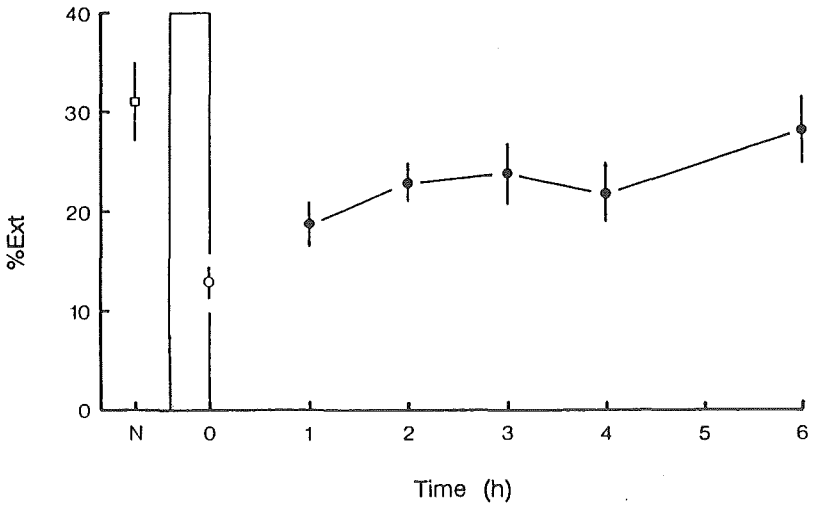

Fig. 2.13 Changes in oxygen extraction efficiency (%Ext) during recovery from progressive hypoxia (●). Vertical bar represents the hypoxic period, N = normoxia (□), 0h = the end of hypoxia (○). Mean ± 1 SEM, n = 5.

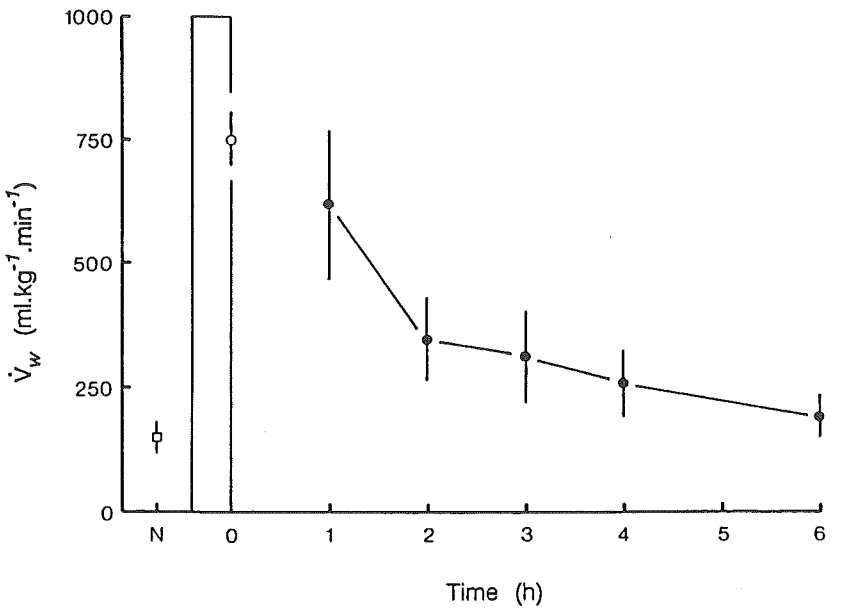

Fig. 2.14 Changes in $\dot{V}_w$ during recovery from hypoxia. The period of hypoxia is represented by the vertical bar. N = normoxia, 0h = the value determined at the end of hypoxia. Data are given as mean ± 1 SEM, n = 5. Symbols as in Fig. 2.12.

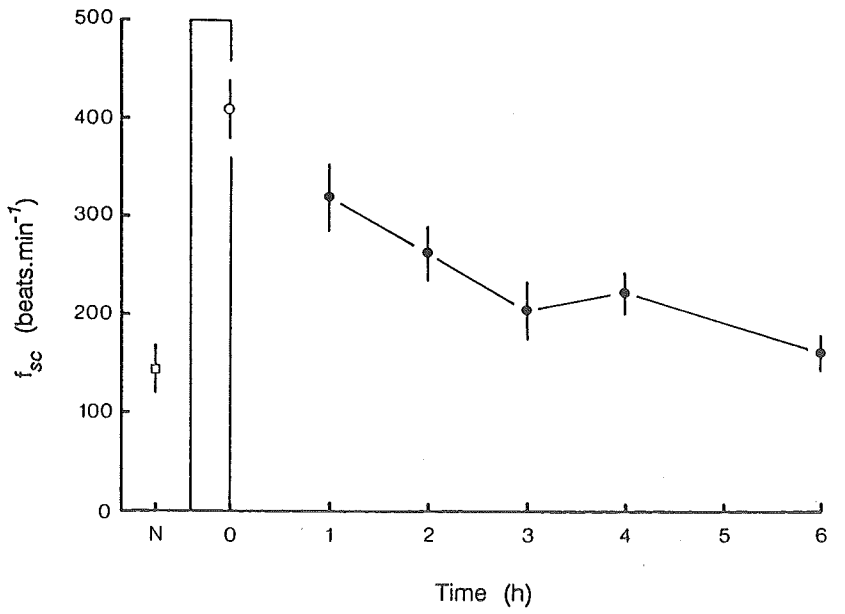

Fig. 2.15 Ventilation frequency (f_{sc} = sum of the left and right scaphognathites) in normoxia (N), at the end of hypoxia (0h) and over 6h in normoxic water. Vertical bar represents 2 - 4h hypoxia. Data are given for n = 4 animals as mean ± 1 SEM. Symbols as in Fig. 2.12.

animal (Fig. 2.16). Calculations of the ventilatory requirement, $\dot{V}_w/\dot{M}O_2$, showed an rapid decrease from the 150 ml.μmol O_2^{-1} used at 40 Torr to about 25 ml.μmol O_{2-1} after 1h.

Least squares regression was performed on the relationship between f_{sc} and $\dot{V}_w$ from the means of all data obtained in this chapter (Fig. 2.17). The intercept (-200) indicates that scaphognathite stroke volume increases with increasing ventilation frequency. The relationship between respiratory frequency and volume, with an r^2 value of 0.91 was highly significant ($p<0.0004$). Within the range of mean scaphognathite frequencies (~140 - 410 bpm) scaphognathite stroke volume varied between 1.2 and 1.8 ml.beat^{-1}.

DISCUSSION

Acclimation

Jasus, like the lobster *Homarus* (Butler et al., 1978: McMahon et al., 1978) and the crayfish *Orconectes rusticus* (McMahon et al., 1974) requires at least 48h in normoxic water to recover from the effects of air exposure and handling. Oxygen consumption, ventilation volume and respiratory frequency all decreased from the peak levels recorded immediately after the animals were placed into the experimental chamber. Percentage extraction of oxygen from the inspired water increased, although the total change was small. Of the measured variables oxygen consumption was restored most rapidly, while ventilation frequency showed the longest recovery response.

The settled value for oxygen uptake was lower in *Jasus* than those reported for a large number of crustaceans (Table 1). The only comparable level of oxygen uptake to that of *Jasus* occurs in the crayfish *Austropotamobius pallipes*, which has an oxygen consumption of ~11 μmol.kg^{-1}.min^{-1} at the same temperature of 15°C, a surprisingly

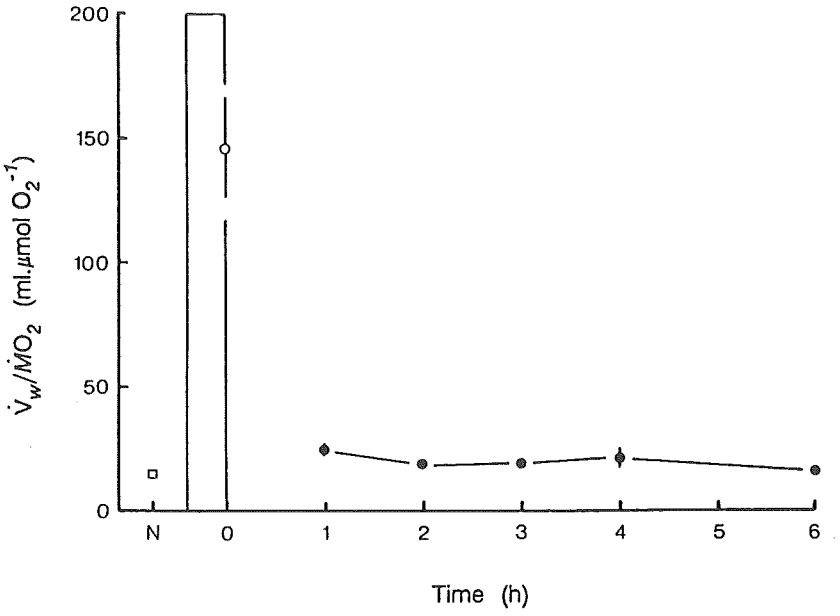

Fig. 2.16 The convection requirement ($\dot{V}_w/\dot{M}O_2$) at rest in normoxia (N), at the end of hypoxia (0h) and during subsequent recovery in normoxic water. The hypoxic period is indicated by the vertical bar. Data = mean ± 1 SEM, n = 5. Symbols as in Fig. 2.12.

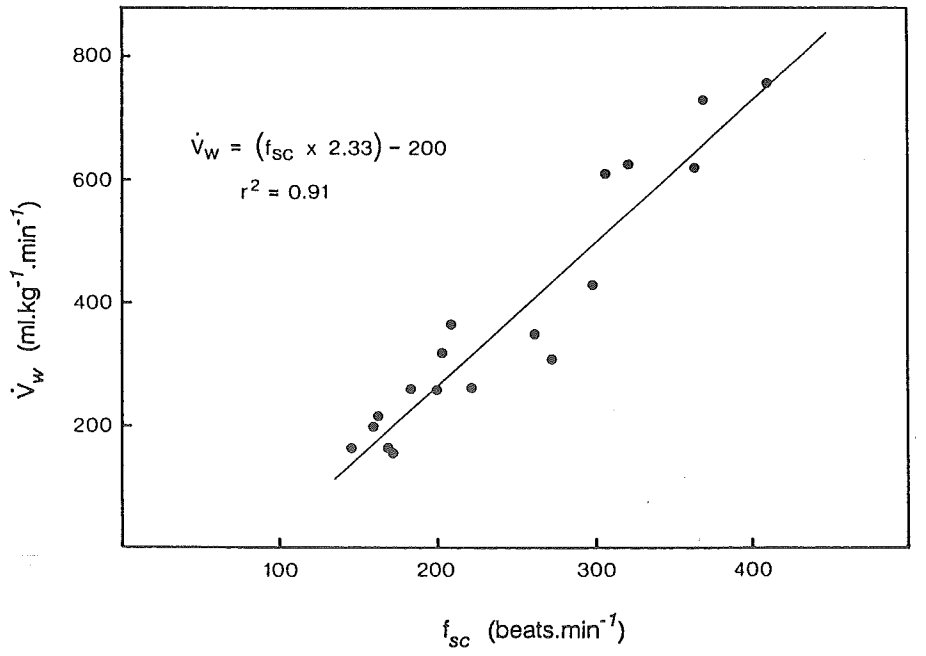

Fig. 2.17 Least squares regression relating ventilation volume and total scaphognathite rate. The data points represent mean values for each data set in this chapter. The relationship between $\dot{V}_w$ and f_{sc} was highly significant ($p<0.0004$).

Table 1. Comparison of oxygen uptake and ventilatory variables in Jasus edwardsii at rest with published values for some other crustaceans.

Species	Weight g	Temp °C	$\dot{M}O_2$ µmol/kg/min	$\dot{V}_w$ ml/kg/min	f_{sc} bpm	%Ext	$\dot{V}_w/\dot{M}O_2$ ml/µmol	Source
Water-breathers								
Jasus edwardsii	300-730	15	10.4	151	144	31	14.8	Present study
Cancer magister	551-960	8	24.6	356	148	28	14.2	McMahon et al., 1979
Cancer productus	>200	10	17[a]	200[a]	73[a]	32[b]	11.2[b]	DeFur and McMahon, 1984a
Callinectes sapidus	90-190	20	49.6	490	188	50	9.9	Booth et al., 1982
---	150-300	22-28	46	555	196	53	12.1	Batterton and Cameron 1978
Carcinus maenas	64	17-18	~30	578		30	19.3	Taylor et al., 1977
Austropotamobius pallipes	25-80	15	11.2	96	148	39	8.6	Taylor and Wheatly, 1981
Homarus americanus	?	12-15	22.3	487	202	27	22	McMahon and Wilkens, 1975
Homarus vulgaris	220-551	15	17	233	95-300	~41	13.7	Butler et al., 1978
Air-breathers								
Cardisoma carnifex	250-500	25	37.6	50	60	13	1.3	Wood and Randall, 1981b
Cardisoma guanhumi	165.5		26	20		2.8	0.8	Herreid et al., 1979

$\dot{M}O_2$ = weight specific oxygen consumption, $\dot{V}_w$ refers to ventilation in either water or air, f_{sc} = total scaphognathite frequency (sum of the left and right scaphognathites). It was assumed that, unless otherwise stated, literature values were mean rates (f_{sc} = (left + right)/2). %Ext = extraction efficiency in either water or air. $\dot{V}_w/\dot{M}O_2$ = convection requirement. [a] = estimated from data given in figures by the authors, [b] = calculated from data.

low rate for an animal which is considerably smaller than *Jasus* (Wheatly and Taylor, 1981). The rate of branchial ventilation was somewhat higher in *Jasus* than in *Austropotamobius* (151 $ml.kg^{-1}.min^{-1}$ compared to 97 $ml.kg^{-1}.min^{-1}$) which is, at least in part, a reflection of the lower oxygen extraction by the rock lobsters.

Calculations of the convection requirement showed a gradual decrease in $\dot{V}_w/\dot{M}O_2$ with acclimation. The higher pumping rates measured during the first hours of acclimation were reflected in the somewhat depressed values of percent extraction of oxygen. In general terms though, there was relatively little change in either %Ext or $\dot{V}_w/\dot{M}O_2$, indicating good matching between the ventilatory system and oxygen uptake. The initially high rate of ventilation was accompanied by a similarly high respiratory frequency. Both variables generally decreased with time, similar patterns occurring in both. The initially high levels of ventilation at the start of acclimation, as illustrated by the $\dot{V}_w$ and f_{sc} data, would also be expected to influence blood gas tensions, which may in turn alter blood acid-base state. Since $\dot{V}_w$ at 24h was still significantly elevated over the 48h value changes in acid-base state would also be likely at that time. Thus the high values for oxygen uptake and ventilation even 24 hours after handling confirm the need for at least 48h acclimation to the experimental apparatus before experiments are performed.

Hypoxia

Crustaceans exhibit a variety of responses during a decline in oxygen tension. At one extreme some species are able to actively regulate their oxygen consumption down to low levels of inspired oxygen tensions, whereas the oxygen consumption of others is dependent on the PO_2 of the medium. At either extreme animals may be said to be independent or dependent on the ambient oxygen tension.

The present investigation has shown that *Jasus* exhibits both of

these responses. The resting level of oxygen uptake was maintained down to a critical inspired tension of around 80 Torr. Assuming that the resting level of tissue metabolism is maintained, then below 80 Torr oxygen consumption was dependent on P_IO_2. Measurements of $\dot{M}O_2$ between 80 and 30 Torr showed an almost exact linearity with ambient oxygen levels, $\dot{M}O_2$ at 40 Torr being approximately half that at 80 Torr. $P_{(crit)}$ was high compared to that of many other aquatic species which maintain their resting level of oxygen uptake. In *Palaemon elegans*, a species which shows a high degree of respiratory independence, oxygen consumption can be maintained down to 15 Torr (Morris and Taylor, 1985) and critical values of 40-50 Torr and 30-40 Torr have been reported for the crayfish *Austropotamobius* (Wheatly and Taylor, 1981) and the lobster *Homarus* (McMahon and Wilkens, 1975), respectively. Comparison with the data of other authors is hampered by the different methods used to assess $P_{(crit)}$ (either statistical difference from normoxic values or visual observation of a 'break point'). However, examination of the data of these authors by the latter method, which avoids problems associated with the accuracy of measurements, supports the observation that *Jasus* is less oxygen-independent than the other species mentioned above, a factor which may prove limiting to its distribution.

Since P_IO_2, and therefore P_aO_2, decreases during hypoxia, regulation of oxygen uptake depends on improving O_2 conductance between the medium and the animal. Herreid (1980) outlined several ways in which this might be achieved. These mechanisms include increases in gill perfusion, variations in haemocyanin oxygen affinity or ventilatory changes. This latter mechanism has been the most extensively investigated and may involve changes in ventilatory flow rate or oxygen extraction. An increase in branchial water flow is a feature of the regulatory response to hypoxia in many crustaceans (e.g. *Carcinus*, Taylor, 1976; *Austropotamobius*, Wheatly

and Taylor, 1981). In other species, e.g. *Homarus*, regulation is epitomised by maintaining the extraction of oxygen (increasing percentage utilization) while ventilation volume remains more or less constant (Butler et al., 1978).

Jasus provides an interesting contrast to these extremes. Oxygen uptake was regulated down to an inspired PO_2 of ~78 Torr, and was accompanied by an increase in ventilatory flow matched by a qualitatively similar change in ventilation frequency. Percent extraction of oxygen decreased, however, with progressively lower ambient oxygen tensions (Fig. 2.8). This pattern is more reminiscent of the changes occurring during acclimation, when $\dot{V}_w$ and %Ext were inversely related. Similar reciprocal relationships between utilization and ventilation have been recorded for *Carcinus* (Arudpragasam and Naylor, 1964) and *Homarus* (Butler et al., 1978) during settlement, but not during hypoxic exposure. Decreasing extraction efficiency during hypoxia while maintaining oxygen uptake seems inefficient since it relies on pumping larger volumes of water across the respiratory surfaces to support the normal level of oxygen uptake than if %Ext had remained constant. The exact reason responsible for the decrease in extraction efficiency is not known, but implies that there is some barrier to the efficient transfer of oxygen. That $\dot{M}O_2$ fell faster than that of the inspired oxygen tension implies a reduced conductance of oxygen across the gills or a switch to anaerobic metabolism before all of the available oxygen had been used (Herreid, 1980).

By increasing ventilation a greater amount of oxygen was made available at the gills, the amount doubling between 144 and 78 Torr. It was at this latter critical PO_2 that *Jasus* was unable to sustain its resting level of oxygen consumption, $\dot{M}O_2$ becoming dependent on the ambient O_2 tension. Even though more oxygen was available at 50 Torr than at 144 Torr the low levels of extraction determined over

this lower oxygen range were sufficient to cause *Jasus* to lose its respiratory independence.

Increased ventilation was achieved through an increase in ventilation frequency. Although the pattern of changes was similar, the total increase in $\dot{V}_w$ was 1.5 times higher than that of f_{sc}. Thus part of the rise in ventilation volume during hypoxia resulted from an increase in the stroke volume of the scaphognathites (S_v), as shown by the relationship between $\dot{V}_w$ and f_{sc} illustrated in Fig. 2.17. Burggren and McMahon (1983) also found an increase in stroke volume in *Orconectes virilis* at higher ventilation frequencies. They concluded that passive mechanisms reduced S_v at low respiratory frequencies, but that active control mechanisms could be used to increase the stroke volume of the scaphognathites during hypoxia.

Below the critical PO_2 of 78 Torr oxygen uptake was decreasing while f_{sc}, and presumably the metabolic cost of driving the respiratory pump was still slowly increasing. While there was a marked inflection in the rate of rise in f_{sc} at this point, other studies have found that ventilation volume and frequency of ventilation decrease at around the same critical PO_2 as that determined for oxygen consumption (McMahon and Wilkens, 1975; Taylor, 1976; Wheatly and Taylor, 1981). It is possible that, because of the rapidity of the method used to develop hypoxia within the respirometer, the normal ventilatory response to hypoxia was masked, possibly through the animals becoming agitated by the treatment. Additional possibilities are that:
1) the critical tension below which pumping became inefficient had not been reached, or 2) there is an extremely low metabolic cost involved in the beating of the scaphognathites, or 3) ventilation acted independently of oxygen consumption and rose as a direct response to low oxygen tensions.

Recovery from hypoxia initially elicited an increase in the rate

of oxygen uptake, while ventilation volume and frequency decreased slightly from the maximum values measured at the end of hypoxia. The high initial rates of oxygen uptake were enabled by high pumping rates and by increasing the efficiency with which oxygen was extracted from the inspired water. High rates of oxygen uptake were similarly reported for the lobsters *Homarus vulgaris* and *Homarus americanus* after exposure to hypoxia (Butler et al., 1978 and McMahon and Wilkens, 1975, respectively). The higher levels in both these lobsters were also associated with high ventilation volumes and extraction efficiencies occurring on return to normoxic water.

$\dot{M}O_2$ rose to more than double resting $\dot{M}O_2$ and the mean values remained high over the 6h when measurements were made. Calculations based on the means indicate that *Jasus* used some 1400 μmol O_2 in excess of the resting consumption during the recovery period, indicating a substantial oxygen debt incurred by the animals. This is about 3 times greater than the total amount of oxygen 'lost' during hypoxia (~ 600 μmol.kg^{-1}). Possibly the additional oxygen used during recovery compensated for the higher metabolic cost of pumping or removed metabolites which may have accumulated during hypoxia.

In summary, *Jasus* was able to maintain oxygen uptake down to about 80 Torr through an increase in ventilation which offset the decrease in extraction efficiency. High rates of ventilation and oxygen uptake occurred during recovery, probably to repay the oxygen lost during hypoxic exposure.

CHAPTER 3

CHANGES IN RESPIRATION AND ACID-BASE BALANCE AFTER EXERCISE

ABSTRACT

Haemolymph acid-base status and ventilation were studied in the rock lobster, *Jasus edwardsii*, at rest and after rapid and strenuous exercise (50 tail-flips in <3 min.). Exercise resulted in a mixed respiratory and metabolic acidosis, haemolymph pH falling by about 0.3 units 1h after exercise. The maximum changes in both calculated PCO_2 and lactate were small, PCO_2 increasing by just over 1 Torr after 15 minutes and lactate rising by about 0.5 $mmol.l^{-1}$ by 1h post-exercise. Analysis of the pH-bicarbonate diagram indicated that, while the time course of changes in Δlactate and ΔH^+_m were qualitatively similar, the metabolic acid load at 1h was nearly 8 times higher than the concentration of lactate. A small acid load persisted throughout recovery despite a return of lactate to baseline levels. These results suggest that either metabolically produced hydrogen ions may be preferentially released from the intracellular space while lactate ions are retained or that some other acid is produced during exercise. Recovery of pH was associated with an increase in the concentration of bicarbonate. Haemolymph $[Ca^{2+}]$ did not change during exercise or recovery. These data are consistent with restoration of acid-base balance by exchanges of ions across the gills.

Exercise appeared to be fuelled mainly aerobically, since there was only a small increase in haemolymph lactate concentration, compared to a three-fold increase in oxygen uptake after exercise. This increase was enabled by a 2.3-fold increase in scaphognathite rate and a 40% increase in heart rate. $\dot{M}O_2$ was restored to the pre-

exercise level by 8h, but the heart and scaphognathite rates were back to normal only after 24h recovery. It is suggested that, while the primary function of these organs is to promote oxygen delivery, high rates are involved in restoring haemolymph pH.

INTRODUCTION

Studies on the effects of exercise on the respiratory and acid-base physiology of crustaceans have mainly focused on crabs (eg *Callinectes sapidus*, Booth et al, 1982, 1984; *Cancer magister*, McDonald et al., 1979, McMahon et al.,1979; *Uca pugilator*, Full and Herreid, 1984). There have been relatively few studies on the changes resulting from activity in macrurans. Rutledge and Pritchard (1981) examined the interactive effects of exercise and temperature on oxygen uptake in *Pacifastacus*, and Thomas (1954) gave details of oxygen uptake in *Homarus* after exercise.

Exercise normally induces an increase in oxygen consumption, which in some cases may rise to more than 10 times the resting rate (e.g. *Pacifastacus*, Rutledge and Pritchard, 1981). Ventilation is also reported to increase with exercise, while heart rate may either decrease (Herreid et al., 1979) or, more usually, increase (Wood and Randall, 1981b; Houlihan and Innes, 1984; McMahon et al., 1979). Depending on the level of exercise, aerobic respiration may be insufficient to sustain the metabolic rate required, and often there is a shift towards anaerobic metabolism.

Studies of acid-base state have shown a decrease in haemolymph pH accompanying exercise, with both respiratory and metabolic components to the acidosis. Several hours of recovery may be required before the levels of metabolites, blood gases and haemolymph pH are restored to normal, these processes taking considerably longer in

crustaceans than in mammals.

There is a growing number of reports, which include studies on crustaceans (Booth et al., 1984; Greenaway et al., 1988), fish (Piiper et al., 1972; Wood et al., 1977; Perry et al., 1985) and amphibians (Boutilier, et al., 1980; McDonald et al., 1980a), that lactate and hydrogen ions do not appear in the blood in equimolar amounts. Generally blood lactate is higher than the metabolic acid load, although the reverse also occurs. This suggests that different mechanisms are responsible for the release of lactate and hydrogen ions. A number of crustacean studies have shown that lactate opposes the Bohr shift of the haemocyanin molecule associated with a depression of pH. Thus, different release dynamics might be reflected in changes in the oxygen transporting properties of the haemolymph (e.g. Truchot, 1980; Bouchet and Truchot, 1985).

Exercise effects on acid-base and respiration have been reasonably common studies in crustaceans, but most protocols have utilised walking or running or, occasionally, swimming, of the animals. Tail-flipping is a form of activity characteristic of macrurans. This rapid and repetitive flexion of the powerful tail muscles is usually elicited as an escape response. In contrast to other forms of activity, continuous tail-flipping is almost certainly not sustainable. There are few reports on its physiological effects. Rutledge (1981) and Rutledge and Pritchard (1981) examined the respiratory response to tail-flexion using *Pacifastacus leniusculus* and Phillips et al. (1977) studied the influence of this form of exercise on haemolymph lactate levels using various crustaceans, including the Australian yabbie, *Cherax destructor* and the lobster *Homarus gammarus*.

The aim of this study was to determine the influence of exercise on cardiorespiratory variables and acid-base status of the haemolymph of *Jasus edwardsii*. Responses of *Jasus* are compared with those of

other crustaceans. A further objective was to isolate the effects of this perturbation to aid in the interpretation of the effects of emersion (Chapter 4) and exercise and recovery in air (Chapter 5).

MATERIALS AND METHODS

Rock lobsters, weighing between 320 and 610 g, of either sex, were captured off the Canterbury coast of New Zealand, mainly at Akaroa (43°53'S, 172°58'E) and Motunau (43°03'S, 173°04'E). The animals were transported to the laboratory and were maintained for at least 3 weeks before experiments. They were held in recirculating sea water (temperature = 17 ± 1°C, salinity ~35°/oo) and were fed regularly with mussels. Food was withheld for a week before experiments were conducted. All of the animals used had hardened carapaces and were judged to be in intermoult.

Experimental procedure and analytical methods

Exercise in these animals consisted of tail-flipping. Each animal was stimulated into performing approximately 50 tail-flips by either chasing with a stick or by lightly holding the antennae. This level of exercise was considered severe, since the intensity of the response decreased considerably the more tail-flips were performed. The duration of exercise varied, but generally ranged from about 45 seconds to 3 minutes. Changes in physiological variables were measured over the subsequent 48h recovery period. During the experiments the chambers were covered with sheets of black plastic which were arranged to minimise visual disturbances, while permitting the experimenter to view the animals. All experiments were conducted at 17 ± 1°C.

The cardiorespiratory and acid-base variables of the animals

were measured in three series of experiments. Each animal was settled for at least 48h before exercise to establish resting values for the variables measured and to permit recovery from handling effects and electrode implantation. In Series I oxygen consumption was measured on 9 animals. In Series II heart and scaphognathite recordings were made on 7 animals. In Series III pre-branchial haemolymph pH, total CO_2, lactate, calcium and osmotic pressures were measured on a total of 18 animals.

Oxygen uptake ($\dot{M}O_2$) was measured using the closed box method described in Chapter 2. Briefly, this method involved enclosing the animal in a sealed chamber thermostatted to 17 ± 1°C. A sample of sea water was removed from the chamber and the volume (about 5 ml) replaced. The change in inspired PO_2 was measured over a known time interval (normally between 8 - 13 min) with a Strathkelvin PO_2 electrode (1302). The inspired oxygen tension never fell below 120 Torr. $\dot{M}O_2$ was measured at 15 min, and at 1, 2, 4, 8, 24 and 48h after exercise, and calculated using the equation given in Chapter 2.

Heart rate (f_H) and total scaphognathite rate (f_{sc} = sum of the left and right scaphognathites) were measured using the impedance method described previously. Silver electrodes were implanted either side of the heart or scaphognathites, and the resulting impedance signals were detected by Strathkelvin impedance couplers and recorded on a Gould recorder.

A total of 18 rock lobsters were used for measurements of haemolymph acid-base status. The mean for each time interval during exercise and recovery represents data obtained from 8 animals. Preliminary experiments indicated that serial haemolymph sampling caused a progressive decrease in pH. Each animal was therefore sampled only once during each experimental run. After 48h recovery, the lobsters were subjected to further exercise tests, and sampled at different times. Thus each animal used in this series was exercised

and recovered several times. Control (pre-exercise) values and the final recovery values were similar, suggesting that this protocol had no long term effect on haemolymph acid-base characteristics.

Pre-branchial haemolymph (~0.5 - 0.8 ml) was sampled with a 20 gauge needle via the arthrodial membrane at the base of a walking leg. A small volume of haemolymph was withdrawn immediately prior to removal of the main sample, and used to displace the dead space in the syringe. The time between removal of the first and second samples was less than 15 sec. Subsamples were analyzed for pH, total CO_2 (CCO_2), lactate, calcium, osmotic pressure and ammonia content. Haemolymph pH was measured on 70 μl subsamples drawn into a water-jacketed capillary microelectrode (Radiometer G297/G2) thermostatted to 17 ± 1°C and displayed on a pH meter (Radiometer PHM 84). The pH electrode was calibrated to ±0.005 units with precision buffers (Radiometer S1500 and S1510), corrected for temperature. Total CO_2 was measured using the method of Cameron (1971). The cell contained 10 mmol.l^{-1} HCl to which 5 mmol.l^{-1} NaCl had been added and was thermostatted to 40°C to reduce the response time. Twenty microlitre subsamples were bracketed between 20 μl standards (10 mmol.l^{-1} $NaHCO_3$). The change in PCO_2 was measured with a PCO_2 electrode (Radiometer E5036) connected to a Radiometer pH meter (PHM 84).

Pre-branchial lactate concentrations were determined enzymatically using a modified Boehringer food analysis kit for lactate (Cat. No. 139 084). The glycylglycine buffer was modified by decreasing the pH from 10 to 9 using 1.0N HCl and by adding 10 mmol.l^{-1} EDTA, according to the modifications outlined by Engel and Jones (1978) and Graham et al. (1983) for the improvement of lactate assays using UV methods. Haemolymph samples (150 μl) were initially deproteinated by the addition of 300 μl of ice-cold, 0.6 mol.l^{-1} perchloric acid and centrifuged. Either 50 or 150 μl

samples of the supernatant were used in the assay, depending on the expected concentration of lactate. The samples were incubated at 30°C both before and after the addition of LDH, and the change in absorption as NAD was converted to NADH was determined at 340nm (Kontron Uvikon 860 Spectrophotometer) using lithium lactate standards.

Pre-branchial calcium concentrations and osmotic pressures (OP) were measured using frozen haemolymph samples. Measurements made on fresh and frozen samples showed that freezing made no difference to either $[Ca^{2+}]$ or OP. Calcium was measured on 10 μl samples added to 2.0 ml of 0.5% $SrCl_3$ and analyzed with a Varian Techtron 1200 atomic absorption spectrophotometer. Haemolymph osmotic pressure was determined on 8 μl samples with a Wescor 5100C vapour pressure osmometer.

Twenty microlitre aliquots of pre-branchial haemolymph were tested for ammonia using a Boehringer test kit for ammonia and urea, (Cat. No. 124 770) which is based on the phenol/hypochlorite method of Solorzano (1969). The final sample was incubated at 37°C and the absorbance determined at 640nm against a distilled water blank with a Kontron Uvikon 860 spectrophotometer. Ammonium sulphate standards in the appropriate range were also used.

Non-bicarbonate buffer lines were determined *in vitro* for the haemolymph of 6 animals. Each sample was equilibrated with known mixtures of carbon dioxide in air, delivered by a Wösthoff gas mixing pump. The pH and total CO_2 content of the haemolymph were then measured at each CO_2 tension using the methods described previously.

Calculations

(Equation 3.1):

$$PCO_2 \text{ (Torr)} = \frac{CCO_2}{\alpha CO_2 \times (1 + 10^{(pH - pK1)} + 10^{(pH - pK1)} \times 10^{(pH - pK2)})}$$

(Equation 3.2):

$$[HCO_3^- + CO_3^{2-}] \text{ (meq.l}^{-1}\text{)} = \alpha CO_2 . PCO_2 . \{10^{(pH - pK1)} + 2(10^{(pH - pK1)} . 10^{(pH - pK2)})\}$$

(Equation 3.3):

$$\beta \text{ slope of the non-bicarbonate buffer line (meq.l}^{-1}\text{.pH unit}^{-1}\text{)} = \Delta[HCO_3^- + CO_3^{2-}]/\Delta pH$$

(Equation 3.4):

$$\Delta H^+_m \text{ (meq.l}^{-1}\text{)} = [HCO_3^- + CO_3^{2-}]_f - [HCO_3^- + CO_3^{2-}]_i - \beta(pH_f - pH_i)$$

(Equation 3.5):

$$\Delta La^- \text{ (meq.l}^{-1}\text{)} = [La^-]_f - [La^-]_i$$

The value of the solubility coefficient (αCO_2 = 0.0449 mmol.l^{-1}.Torr^{-1}) and for the first and second apparent dissociation constants (pK_1 = 6.013 and pK_2 = 9.29) were determined from the alignment nomograms of Truchot (1976). The 'i' and 'f' of equations 3.4 and 3.5 refer to the values of $[HCO_3^- + CO_3^{2-}]$ and $[La^-]$ before and after exercise, respectively.

Statistical Treatment

All data are presented as the mean ± 1SEM. The data was

initially compared with a General Linear Model ANOVA, and statistical differences between means compared with Fisher's Least Significance Difference test. Significance was designated at $p<0.05$.

RESULTS

Patterns of Heart and Scaphognathite Beating

The normal pattern of resting ventilation in *Jasus edwardsii* is bilateral, usually asynchronous, forward pumping (Fig 3.1a). On occasion one side may cease activity altogether, and even more infrequently both scaphognathites may pause simultaneously (a respiratory pause), although the time spent in this latter mode was of relatively short duration (each pause usually lasting less than 30 sec).

Simultaneous recordings of the activity of the two scaphognathites and the heart were not made. It was observed, however, that a slight bradycardia was always associated with a parallel reduction in the activity of the scaphognathite monitored (Fig. 3.1b). A pause in scaphognathite beating, however, was not always accompanied by a pause in cardiac beating.

By 15 minutes post-exercise, bilateral ventilation invariably occurred and both scaphognathites were beating at approximately the same rate (Fig. 3.1c). with no evidence of either respiratory or cardiac pauses (Figs. 3.1c and 3.1d).

Ventilatory Variables

The rates of oxygen consumption ($\dot{M}O_2$), ventilation frequency (f_{sc}) and heart rate (f_H) at rest, immediately following exercise of 50 tail flaps in water and during the subsequent recovery in water, are shown in Figs. 3.2-3.4.

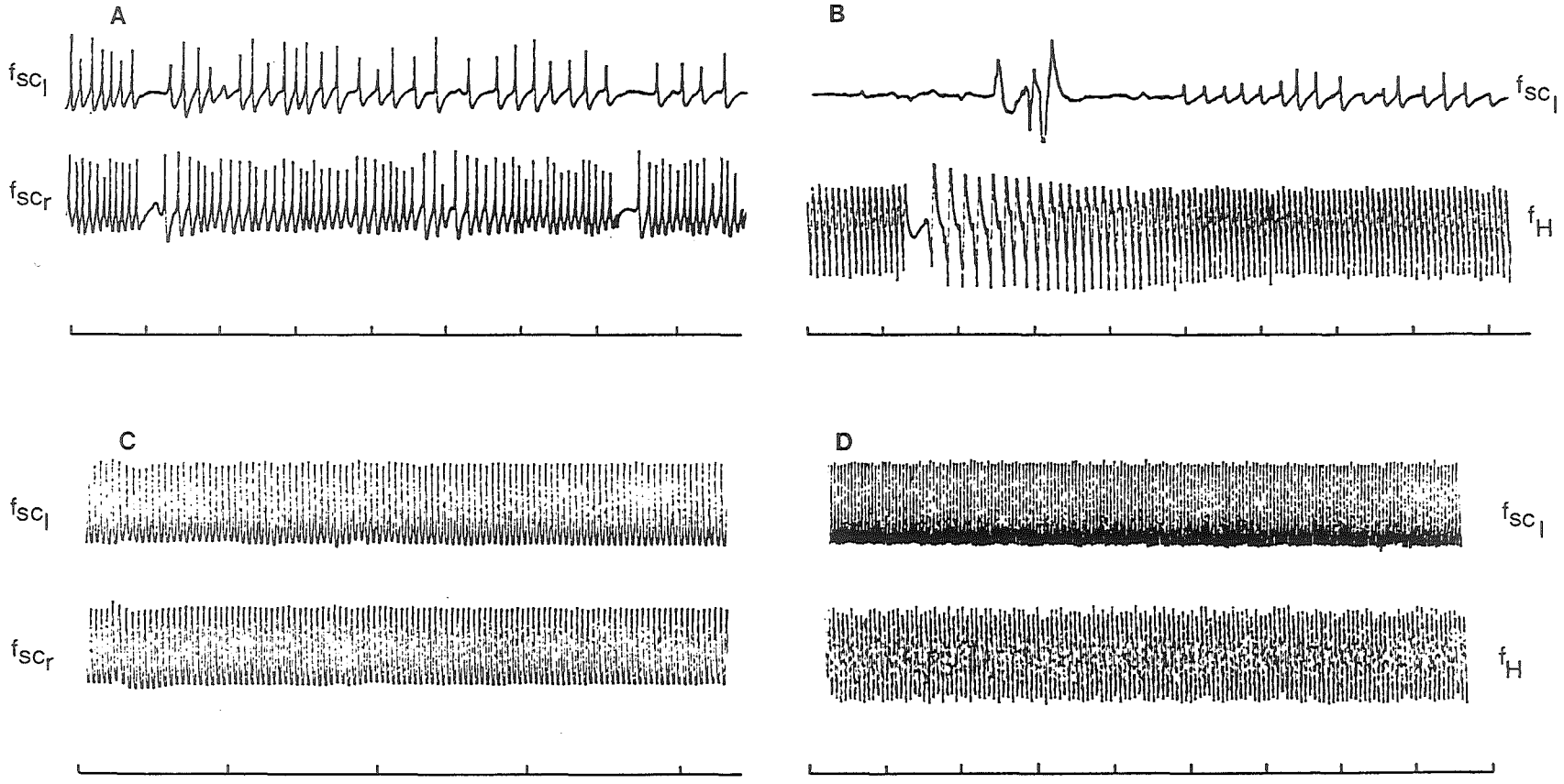

Fig. 3.1 Representative impedance recordings of heart and scaphognathite activity in *Jasus edwardsii* at 17°C. Figs. A and B = rest, C and D = 3 min. post-exercise. Figures A and C show simultaneous recordings of the left (top trace) and right (bottom trace) scaphognathites. Figures B and D show the activity of the left scaphognathite (top) and heart (bottom). Time base = 10 sec. Further details in the text.

In control, settled, *Jasus* resting oxygen consumption was 13.92 ± 1.39 $\mu mol.kg^{-1}\ min^{-1}$ (Fig. 3.2). On completion of the 50 tail-flips' exercise there was a large and rapid increase in $\dot{M}O_2$ to a measured peak of 38.73 ± 1.83 $\mu mol.kg^{-1}\ min^{-1}$ at 15 minutes post-exercise, a highly significant ($p<0.001$) increase of about 300% in oxygen uptake. With further recovery the rate gradually returned to baseline levels. The reduction in $\dot{M}O_2$ occurred most rapidly in the first hour and then more slowly, but oxygen uptake was back to a level not significantly different to resting $\dot{M}O_2$ by 8h post-exercise.

Mean resting f_{sc} and f_H were 138 ± 20 bpm and 58.6 ± 6.7 bpm, respectively (Figs. 3.3 and 3.4). Ventilation frequency more than doubled after exercise, peaking at 363 ± 20.2 bpm at 15 minutes post-exercise. A tachycardia also developed after exercise, but the maximum rate was reached slightly later than those of either ventilation frequency or oxygen uptake, the peak being measured at 1h post-exercise. In addition, the relative increase in f_H was less than for f_{sc} or $\dot{M}O_2$. The highest recorded mean value of 80 bpm at 1h represented a 35% increase over the pre-exercise level.

Subsequent recovery of f_{sc} and f_H showed similar time courses. Both variables dropped away from their respective peaks to temporary plateaux between 4-8h, although both variables were not significantly different from their control values by 4h post-exercise.

Acid-Base Variables

The influence of tail-flipping on pre-branchial pH, calculated PCO_2, bicarbonate ($[HCO_3^- \pm CO_3^{2-}]$), [lactate] and [ammonia] in pre-branchial haemolymph is shown in Figs. 3.5-3.9. In settled rock lobsters at 17°C haemolymph pH was 7.53 ± 0.03 units, calculated PCO_2 levels were approximately 2 Torr, haemolymph bicarbonate about 3.3 $meq.l^{-1}$ and haemolymph lactate concentration approximately 0.14 $mmol.l^{-1}$. When *Jasus* was exercised changes were observed in all these

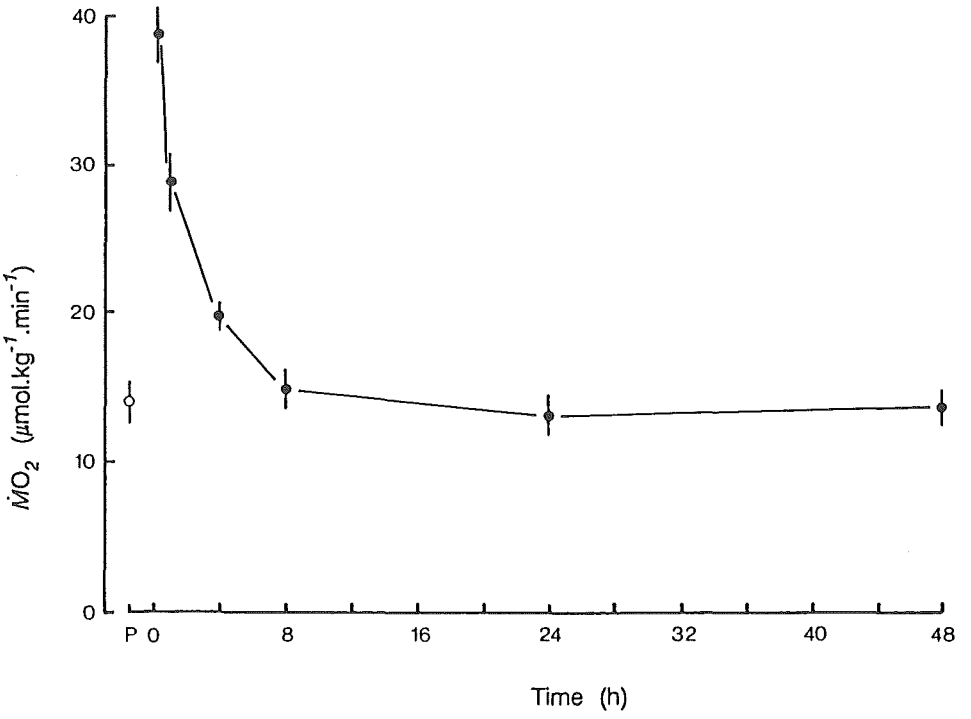

Fig. 3.2 Changes in oxygen uptake following brief, strenuous exercise in *Jasus* at 17°C. Open circle represents $\dot{M}O_2$ immediately prior to exercise (P), closed circles represent post-exercise $\dot{M}O_2$. 0h = immediately post-exercise. Data are given as mean ± 1 SEM, n = 9.

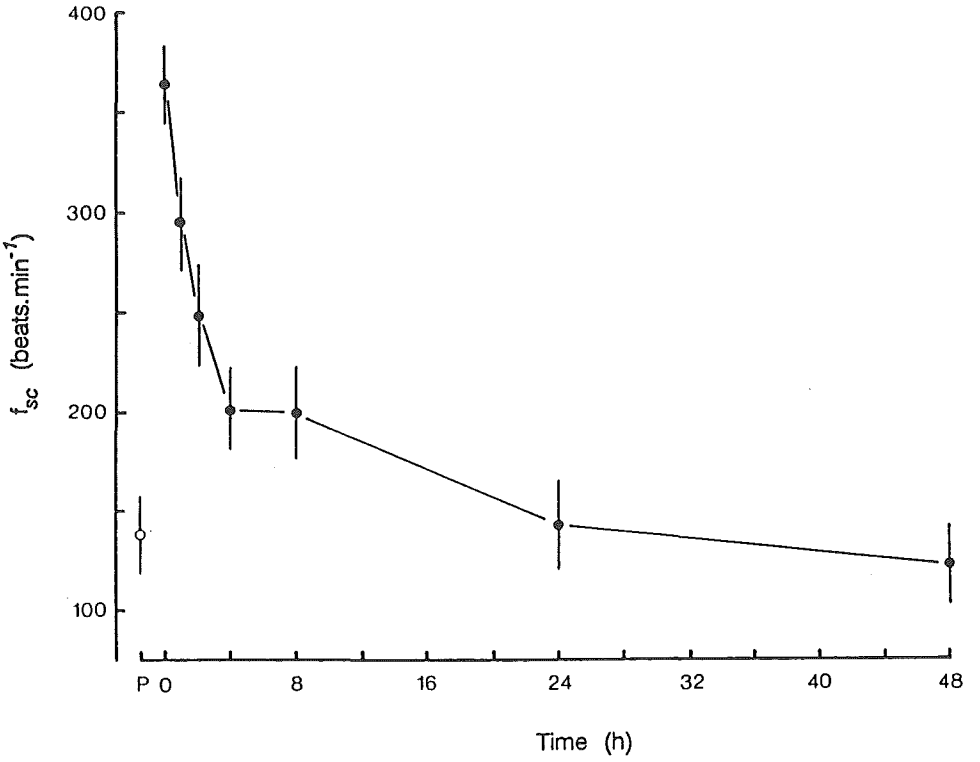

Fig. 3.3 The effect of exercise on scaphognathite activity (f_{sc} = sum of the left and right scaphognathites) in *Jasus* at 17°C. n = 7. Other details as in Fig. 3.2.

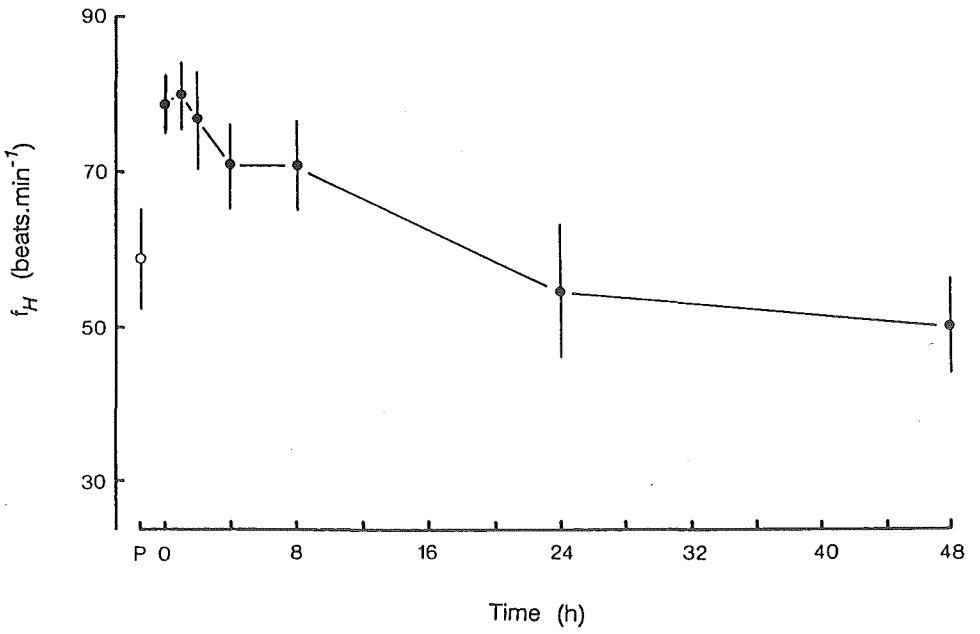

Fig. 3.4 Changes in heart rate (f_H) after brief, strenuous exercise at 17°C. n = 7. Other details as in Fig. 3.2.

variables. Haemolymph pH dropped sharply, mainly within 15 minutes post-exercise, but decreased further to a low of 7.183 ± 0.033 units at 1h post-exercise (Fig. 3.5). Thereafter pre-branchial pH levels were quickly restored to baseline levels and were not significantly different from the resting pH by 8h post-exercise.

There was a small, but significant ($p<0.001$) change in haemolymph PCO_2 on exercise (Fig. 3.6). PCO_2 rose by approximately 1.2 Torr within the first 15 minutes of recovery. A small decrease from the 15 min. value was observed at 1h post-exercise, but the CO_2 tension was still significantly higher than the control value at that time ($p<0.05$). By 8h PCO_2 was not statistically different from the baseline tension.

Mean haemolymph bicarbonate (Fig. 3.7) fell after exercise from the control concentration of 3.34 ± 0.41 meq.l^{-1} to 2.18 ± 0.23 meq.l^{-1} after 1h ($p<0.05$). Variability in bicarbonate was relatively high and none of the other values was significantly different from the controls. However, mean values suggest that restoration of haemolymph bicarbonate may have been rather slower than either haemolymph pH or PCO_2.

Although there was a only a small increase in [lactate] in absolute terms after exercise (0.4 mmol.l^{-1}, statistically significant at 1h, $p<0.001$, Fig. 3.8), the change represented a 4-fold rise over the resting concentration. With further recovery the concentration was reduced and was not significantly different from the resting levels by 8h post-exercise.

Haemolymph ammonia (Fig. 3.9) levels were highly variable. At 1h the concentration was 0.72 ± 0.04 mmol.l^{-1}, which was significantly higher than the resting concentration of 0.60 ± 0.10 mmol.l^{-1} ($p<0.05$). None of the other changes was significantly different from the resting concentration.

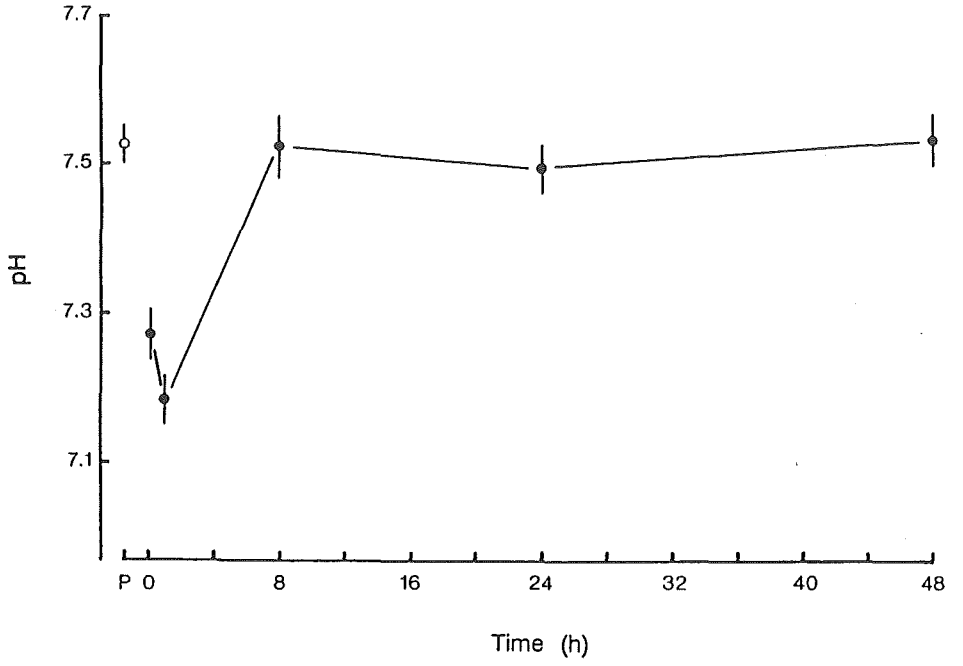

Fig. 3.5 Pre-branchial haemolymph pH at rest (P), and following brief, but strenuous, exercise at 17°C. n = 8. Other details as in Fig. 3.2.

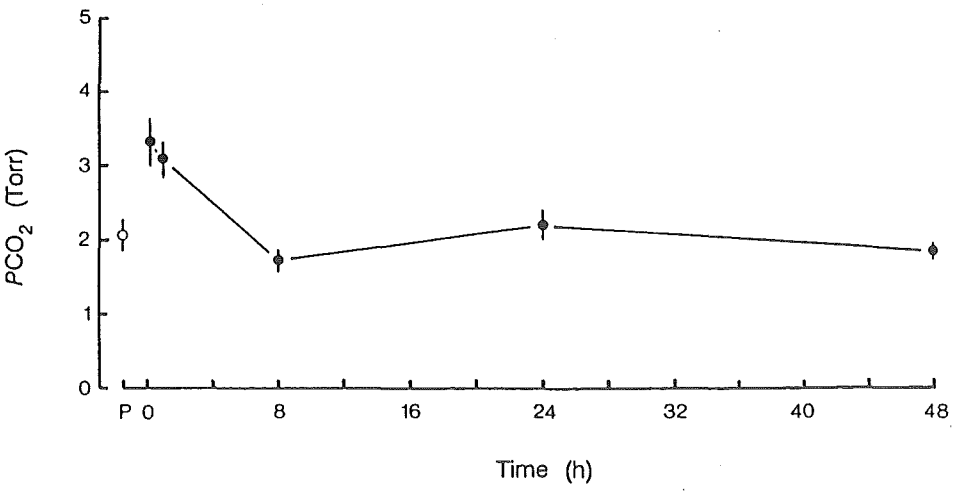

Fig. 3.6 The effect of exercise on calculated PCO_2 of pre-branchial haemolymph in *Jasus edwardsii* at 17°C. n = 8. Other details as in Fig. 3.2.

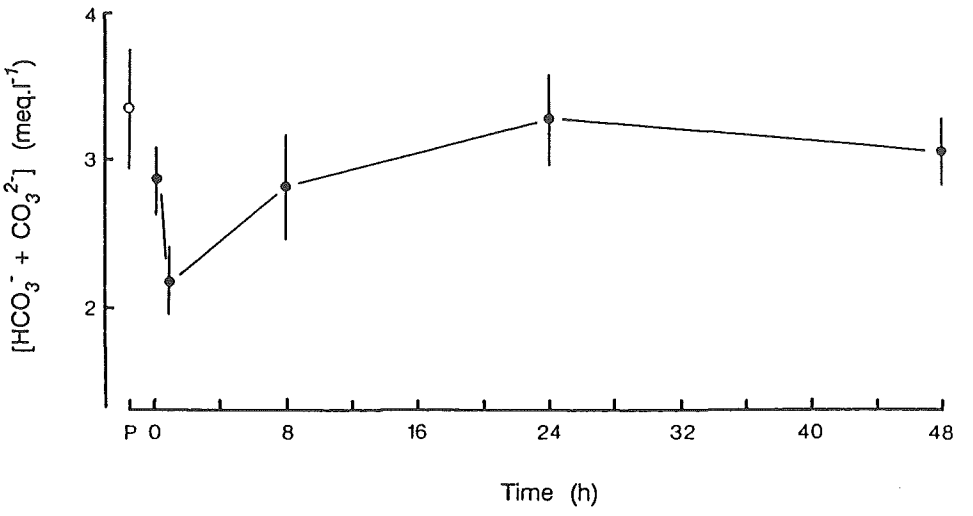

Fig. 3.7 Changes in pre-branchial bicarbonate concentration ($[HCO_3^- + CO_3^{2-}]$) at rest and following exercise at 17°C. n = 8. Other details as in Fig. 3.2.

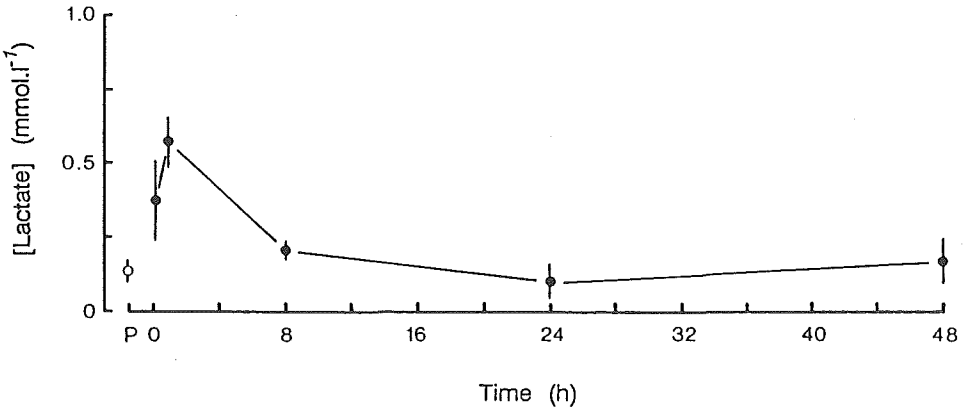

Fig. 3.8 Haemolymph lactate concentration at rest and following exercise in *Jasus* at 17°C. n = 8. Other details as in Fig. 3.2.

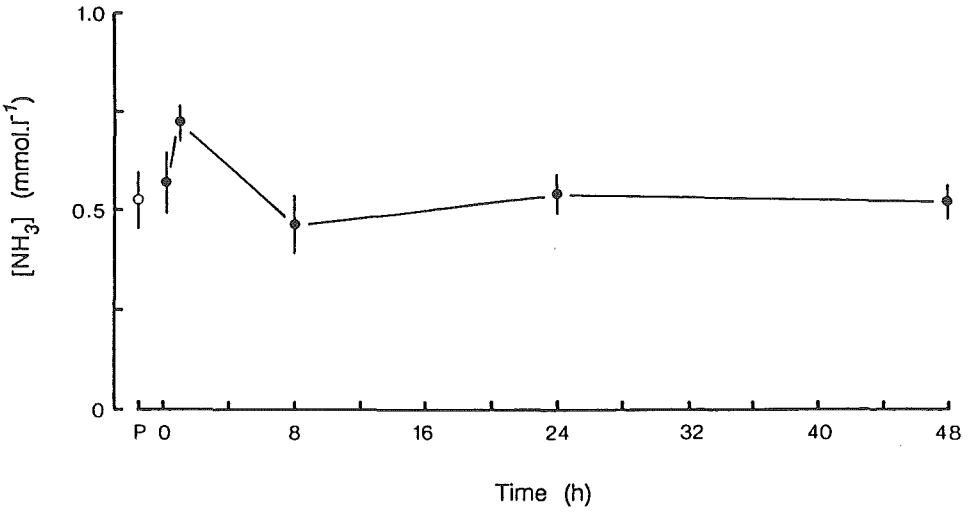

Fig. 3.9 Changes in haemolymph ammonia (NH_3 + NH_4^+) resulting from exercise of 50 tail-flips in water. n = 8. Other details as in Fig. 3.2.

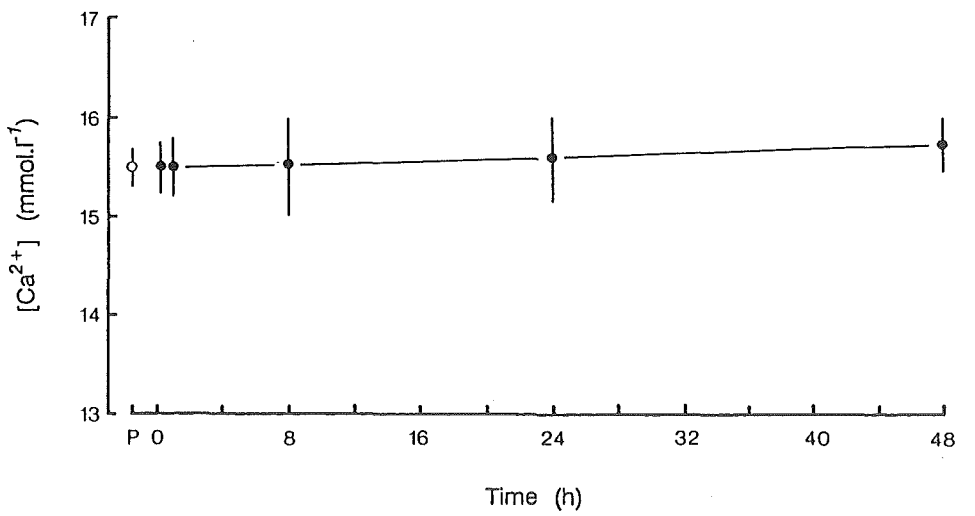

Fig. 3.10 Pre-branchial haemolymph [Ca^{2+}] at rest in water and following exercise at 17°C. n = 8. Other details are the same as those given in Fig. 3.2.

Haemolymph Calcium and Osmotic Pressure

The concentration of calcium in the haemolymph of *Jasus* before and after exercise is depicted in Fig. 3.10. While there was a very slight (0.25 $mmol.l^{-1}$) overall increase in mean haemolymph calcium over the 48h recovery period, at no time were these values significantly different from the resting concentration of 15.49 ± 0.20 $mmol.l^{-1}$.

Changes in haemolymph osmotic pressure were small, varying by no more than 1% from the pre-exercise value of 1015 ± 7 $mOsm.kg^{-1}$. None of the changes was statistically significant ($p>0.05$).

Acid-Base Analysis

Measurements of haemolymph pH and bicarbonate at different external PCO_2 levels yielded an *in vitro* buffer line which had a mean slope of -8.03 $meq.l^{-1}.(pH\ unit)^{-1}$. Fig. 3.11 depicts the sequential changes occurring in pH, $[HCO_3^- + CO_3^{2-}]$ and PCO_2 in *Jasus* at rest and during recovery from exercise, together with the non-bicarbonate buffer line. Using the techniques outlined by Wood et al. (1977) and Davenport (1974), and calculating on the changes in $[H^+]$, the diagram reveals that the acidosis observed at 15 minutes post-exercise was of both metabolic and respiratory origins, with metabolic acids responsible for the major portion of the acidosis (between 65 - 70%) and the remainder from respiratory (CO_2) sources. The metabolic acids increased further into recovery and exerted a proportionally greater influence on the pH observed at 1h post-exercise. By 8h both the metabolic and respiratory acidoses had been considerably reduced over the 1h levels, and despite the persistence of a very small metabolic acidosis there was even a slight respiratory alkalosis observed at that time. Later in recovery these two components were so balanced as to restore haemolymph pH to within 0.006 units of the pre-exercise pH.

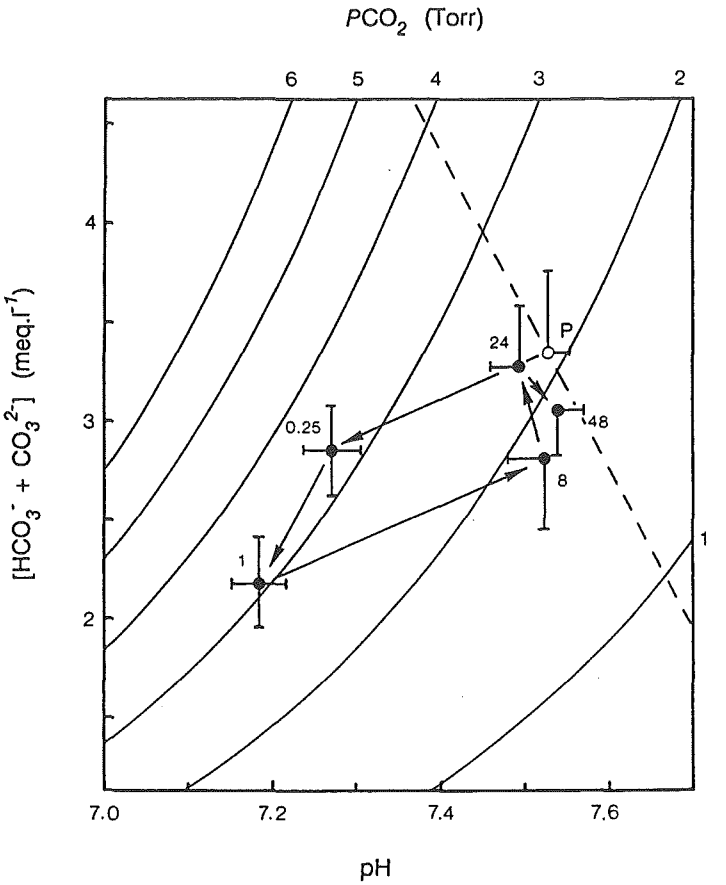

Fig. 3.11 pH-bicarbonate diagram showing simultaneous changes in haemolymph pH, $[HCO_3^- + CO_3^{2-}]$, and PCO_2 after exercise. The sampling time is given beside each point. Open symbol and P = pre-exercise. The dashed line (— — —) represents the non-bicarbonate buffer line determined *in vitro* on haemolymph obtained from 6 settled animals.

DISCUSSION

Patterns of Heart and Scaphognathite Beating

Jasus conforms to the general ventilatory pattern shown by the majority of crustaceans which employ forward pumping as the normal mode of ventilation. Unilateral ventilation and simultaneous pauses of both scaphognathites have been well documented for a number of crustaceans, including the brachyurans *Cancer magister* (McDonald et al., 1977), *Cancer productus* (McMahon and Wilkens, 1977) and *Carcinus maenas* (Jouve-Duhamel and Truchot, 1985), and the macrurans *Homarus americanus* (McMahon and Wilkens, 1972), *Homarus vulgaris* (Butler et al., 1978) and *Orconectes rusticus* (Wilkes and McMahon, 1982).

Many of the authors cited above have speculated on reasons for these patterns. In both *Homarus* (Butler et al., 1978) and *Cancer* (McDonald et al., 1977) unilateral ventilation is the norm at rest under normoxic conditions and both authors have suggested that ventilation of one branchial cavity is sufficient to sustain metabolic requirements, thus minimising the water convection requirement ($\dot{V}_w/\dot{M}O_2$). However, unilateral ventilation occurred to a far lesser extent in *Jasus* than in these other crustaceans.

In addition, although pre-exercise $\dot{M}O_2$ was low compared to most aquatic crustaceans, the total rate of beating of the scaphognathites was relatively high. This implies that either: 1) extraction efficiency (%Ext) is low, and by extension $\dot{V}_w/\dot{M}O_2$ is high; or 2) the stroke volume (S_v) of the scaphognathites is low. It does not seem likely that *Jasus* differs appreciably from other aquatic crustaceans in extraction efficiency, since the experiments in Chapter 2 showed that mean %Ext was about 30% and in some individuals more than 50% of the available oxygen could be extracted from the inspired water. Measurements of ventilation volume ($\dot{V}_w$) in Chapter 2 indicated that

while $\dot{V}_w$ was generally less than that reported for many crustaceans, oxygen uptake was also lower. Thus the ratio of $\dot{V}_w$ to $\dot{M}O_2$ lies within the range shown by other crustaceans. However, estimates of scaphognathite stroke volume indicated that between 1-2 ml of water is passed through the gills for each beat of the scaphognathite. This is somewhat lower than that of various other crustaceans (eg 2-6 ml/beat in *Orconectes virilis*, Burggren and McMahon, 1983; 2-3 ml/beat in *Cancer magister* (McMahon et al., 1979) and *Cancer productus* (deFur and McMahon, 1984a); 4ml/beat in *Carcinus maenas*, Wilkens et al., 1984).

During the recording of the activity of the heart and of one scaphognathite, it was noted that a bradycardia was always accompanied by a reduction in the activity of the scaphognathite measured, but that the reverse, a pause or reduced rate of beating of the scaphognathite, was not always associated with a slower heart rate (Fig. 3.1b). This suggests that bradycardia only occurs during reduced bilateral activity of the scaphognathites. This agrees with the findings of a number of authors (e.g. Butler et al., 1978; McDonald et al., 1977; McMahon and Wilkens, 1972, 1975; Taylor et al., 1973) who have reported close matching of the scaphognathites and the heart.

The relationship observed between cardiac and scaphognathite activity is thought to be under common nervous (e.g. Taylor, 1987), hormonal (e.g. Wilkens, 1981; McMahon and Wilkens, 1983) or exogenous control (McMahon and Wilkens, 1977), the latter via changes in circulating O_2 and/or CO_2 levels acting directly on the cardiorespiratory control centres. Co-ordination of the activities of the scaphognathites and heart will match ventilation to perfusion, and therefore ensure the most efficient transfer of gases between the animal and medium.

Variations in Oxygen Consumption, Scaphognathite Frequency and Heart Rate

In *Jasus* the resting level of oxygen consumption is lower than that recorded for a number of other crustacean species, even though the experimental temperature used was slightly higher. In this study resting $\dot{M}O_2$ was measured as 13.92 ± 1.39 μmol $O_2.kg^{-1}.min^{-1}$ at 17°C which was not significantly different to the resting level of oxygen uptake reported for *Jasus* in Chapter 2 ($p>0.1$) Even though it was slightly higher than that reported previously, it is still lower than that of a number of other crustaceans of similar weight (see Table 1, Chapter 2). The low level of resting oxygen consumption found in *Jasus* may be a reflection of the experimental protocol used in this study. The animals were unfettered and were not masked as has been the case in many other experiments. In addition, the rock lobsters were not subjected to any operative procedures prior to their introduction into the respirometers, which would also reduce the initially high stress levels commented on by a number of authors. Since the only disturbance involved in determining $\dot{M}O_2$ was the actual confinement of the animals, this probably is more accurately described as a 'settled' or 'quiescent' rate of oxygen consumption rather than a 'routine' level.

The changes occurring in *Jasus* under the exercise regime used in these experiments (50 tail flaps in <3 minutes) are difficult to compare quantitatively with those of other studies. In this study, *Jasus* was subjected to a high-intensity form of activity. Most other studies present the results of a moderate or heavy workload which, while often exhausting, can nevertheless be sustained for a longer period of time. This distinction is important, since the physiological response is determined by the combined intensity and duration of activity. In high intensity exercise, as typified by the escape response, anaerobic metabolism is considered the main energy

source, whereas sustainable exercise is supported mainly by aerobic metabolism (Bennett, 1978).

These considerations notwithstanding, the general patterns of ventilation, circulation and oxygen uptake are similar in *Jasus* after exercise to those exhibited by other crustaceans. The burst-type exercise used in this study was close to exhausting for the majority of the animals. Oxygen uptake rose from a mean 13.92 $\mu mol.kg^{-1}.min^{-1}$ in settled animals to 38.73 $\mu mol.kg^{-1}.min^{-1}$ after exercise, a 2.8-fold increase in oxygen usage. This measured increase in $\dot{M}O_2$ does not necessarily represent the maximal oxygen uptake achievable ($\dot{M}O_{2(max)}$), since burst-type activity, as indicated above, is usually supported anaerobically. It is possible that $\dot{M}O_{2(max)}$ may be higher than the peak recorded here. However, in later chapters $\dot{M}O_2$ was never measured higher than this value, even under conditions of high oxygen demand (Chapters 4 and 5).

The observed change in $\dot{M}O_2$ is considerably lower than that reported for most crustaceans exercised to exhaustion. Nevertheless, it compares favourably with the increases in $\dot{M}O_2$ reported for *Callinectes sapidus* (2.5 times, Booth et al., 1982) and *Cardisoma carnifex* (2.6 times, Wood and Randall, 1981b). Comparison with these values is complicated by the initial resting rates reported for *Callinectes* and *Cardisoma*, since both species were permitted only a few hours to settle to the experimental apparatus. Thus, the relative increases observed in *Callinectes* and *Cardisoma* could be higher than measured. However, the ability of *Jasus* and other crustaceans to raise oxygen uptake is considerably lower than that of even slow-moving fish (Poulson, 1963), where oxygen uptake can increase by more than 10-fold after exercise. The elasmobranch *Squalus acanthias* exhibits a lesser ability to increase its oxygen consumption, since Brett and Blackburn (1978) reported only a 2.7-fold increase in $\dot{M}O_2$ after heavy exercise.

In absolute terms, the change in $\dot{M}O_2$ (25 μmol.kg^{-1}.min^{-1}) is lower in *Jasus* than that of most crustaceans. Maximal exercise in *Cancer magister* increased oxygen uptake by 45 μmol.kg^{-1}.min^{-1} (McMahon et al., 1979), while the terrestrial crab *Cardisoma guanhumi* has an aerobic scope of 97 μmol.kg^{-1}.min^{-1} (Herreid et al., 1979). If the supposedly anaerobic nature of the exercise protocol is considered, it is perhaps not surprising that *Jasus* showed a low aerobic scope. The changes in haemolymph [lactate] (discussed below) were, however, very small (~ 0.4 mmol.l^{-1}), which does not support the concept that the escape response of *Jasus* is fuelled by anaerobic metabolism.

There are several mechanisms which could enable the $\dot{M}O_2$ increase observed after exercise. Ventilation frequency increased 2.5-fold during the maximal increase in $\dot{M}O_2$. While there is some discussion as to whether scaphognathite stroke volume (S_v) remains constant (see reviews by Wilkens, 1981 and McMahon and Wilkens, 1983), a number of studies have indicated little or no change in S_v, increases in scaphognathite stroke volume of 5.0% and 11% being reported for a doubling of ventilation frequency (Booth et al., 1982; McMahon et al., 1979). Comparison of the $\dot{V}_w$ and f_{sc} data in Chapter 2 also indicated an increase in S_v with increasing scaphognathite frequency. If this relationship holds true during exercise, the observed changes in ventilation frequency will reflect proportionate changes in ventilation volume. Using the relationship between f_{sc} and $\dot{V}_w$ described in Chapter 2, at rest (f_{sc} = 138 bpm) $\dot{V}_w$ was 122 ml.kg^{-1}.min^{-1}, and at 15 minutes post-exercise (f_{sc} = 349 bpm) it had increased to 614 ml.kg^{-1}.min^{-1}. Based on these calculations, the convection requirement increased by 80% after exercise. Since $\dot{V}_w$, $\dot{M}O_2$ and extraction are mathematically related (Herreid, 1980) the measured change in $\dot{M}O_2$ and the calculated increase in $\dot{V}_w$ suggest a large reduction in extraction efficiency immediately post-exercise.

The degree to which %Ext varies after exercise appears to be

species-specific, although reductions of 5-10% are common (see McMahon et al., 1974; Batterton and Cameron, 1978; Butler et al., 1978; McMahon et al., 1979). Logically one would expect a decrease in extraction efficiency, since by increasing branchial water flow, the residence time at the gas exchange surface is reduced, resulting in less time to 'pick up' oxygen molecules.

In addition to the increase in ventilation rate, heart rate also increased following exercise, but to a lesser extent than f_{sc} (about a 35% rise over the settled level). In many crustaceans there is an increase in cardiac output after exercise which mainly results from an increase in stroke volume but also from an increase in frequency (McMahon et al., 1979). The increase in f_H is consistent with increased perfusion of the gills and an improved diffusion gradient for gas exchange. Increasing oxygen delivery to the tissues by raising the volume of water passing through the gills and by increasing perfusion of the gills via the elevation in heart rate are more than sufficient to account for the increase in $\dot{M}O_2$ following exercise.

Oxygen consumption decreased with recovery, albeit slowly, taking 8h to fully return to the settled rate. During the initial stages of recovery changes in oxygen consumption were quite closely correlated with the changes occurring in f_{sc} and f_H, parallel reductions being observed in all three variables. However, by 8h this close matching had broken down since $\dot{M}O_2$ had returned to basal levels but both the heart and scaphognathites pumped at rates elevated over the resting levels by 20% and 45%, respectively. In *Cancer magister* heart rate also remained elevated during recovery from exercise, even when $\dot{M}O_2$ had returned to the pre-exercise level (McMahon et al., 1979). That f_{sc} and f_H at 8h remained at the same rates as their 4h values suggests a reduction in the efficient transfer of gases at this time, although the cause of such an effect is unknown.

Acid-Base Regulation

The energetic demands placed upon *Jasus* under this regime were not able to be met solely through aerobic respiration. Lactate also rose following exercise, although the concentration change in the haemolymph was small (approximately 0.4 $mmol.l^{-1}$) compared to that found in other crustaceans, even taking into account the fact that most other studies report the changes occurring after exhausting exercise. However, even in species undergoing submaximal exercise the haemolymph lactate concentration is considerably higher than that found here. For example, in both *Cardisoma carnifex* (Wood and Randall, 1981a) and *Cyclograpsus lavauxi* (Waldron et al., 1986) lactate rose to approximately 6 $mmol.l^{-1}$ after mild exercise, while Booth et al. (1982) reported that 2 minutes into a moderate exercise regime haemolymph lactate levels in *Callinectes sapidus* had risen to 2.4 $mmol.l^{-1}$. Clearly there are differences in the relative times over which exercise occurred between this study and those of Wood and Randall (1981a) and Waldron et al. (1986) which may account for at least part of the differences in lactate concentration between *Jasus* and these species. However, this cannot explain the different patterns observed in *Jasus* and *Callinectes*, since both represent levels found after ≈2 min. of exercise. The difference may be methodological in origin, or it may be that (1) fuelling of the escape response in *Jasus* is more dependent on increasing aerobic respiration rather than anaerobic metabolism or (2) the haemolymph lactate concentration was undersetimated in *Jasus*.

A metabolic acidosis, attributable in part to the increase in lactate, contributed to the depression of pH. An additional source of hydrogen ions is from the hydration of carbon dioxide in the haemolymph, which rises as a result of an increase in oxygen usage, and possibly through the dehydration of bicarbonate by the action of metabolic acids. Thus, as is shown on the pH-bicarbonate diagram

(Fig. 3.11), the rise in metabolic acid, coupled with elevated levels of PCO_2 was sufficient to depress haemolymph by 0.25 units by 15 minutes post-exercise. The increasing contribution of metabolic H^+ to the acidosis between 15 min and 1h post-exercise is reflected in a similar increase in haemolymph lactate concentration over the same time interval.

Further into recovery both components were reduced; nevertheless, the restoration of haemolymph pH seen at 8h occurred in response to a slight respiratory alkalosis with respect to settled tensions, which offset the small but persistent metabolic acidosis still present in the haemolymph.

Despite a reasonable agreement in qualitative terms between the increases in lactate and PCO_2 with respect to their effects on the $[H^+]$ changes occurring in the haemolymph after exercise, the relationship between lactate concentration and that of metabolically produced hydrogen ions is not so clear cut. The average values for the changes relative to the resting condition (defined as 0) are illustrated in Fig. 3.12. What is clearly apparent is a mismatching between the absolute changes in lactate and hydrogen ions. By 15 minutes post-exercise there was an excess of hydrogen ions over lactate ions of 1.25 $mmol.l^{-1}$, and the gap had widened even further at 1h post-exercise, where nearly 3.5 $mmol.l^{-1}$ could not be accounted for by the lactate concentration present in the haemolymph. Therefore, up to 1h post-exercise ΔH^+_m increased more rapidly than ΔLa^-, but after that time the rate of decline was also more rapid for hydrogen cations than lactate anions.

A similar excess of hydrogen ions over lactate has been remarked on for a number of crustaceans (e.g. *Gecarcinus*, Smatresk et al., 1979; *Birgus*, Smatresk and Cameron, 1981; *Cyclograpsus*, Waldron et al., 1986), for fish (*Platicthys*, Wood et al., 1977) and for the toad, *Bufo marinus* (McDonald et al., 1980a). The acidosis

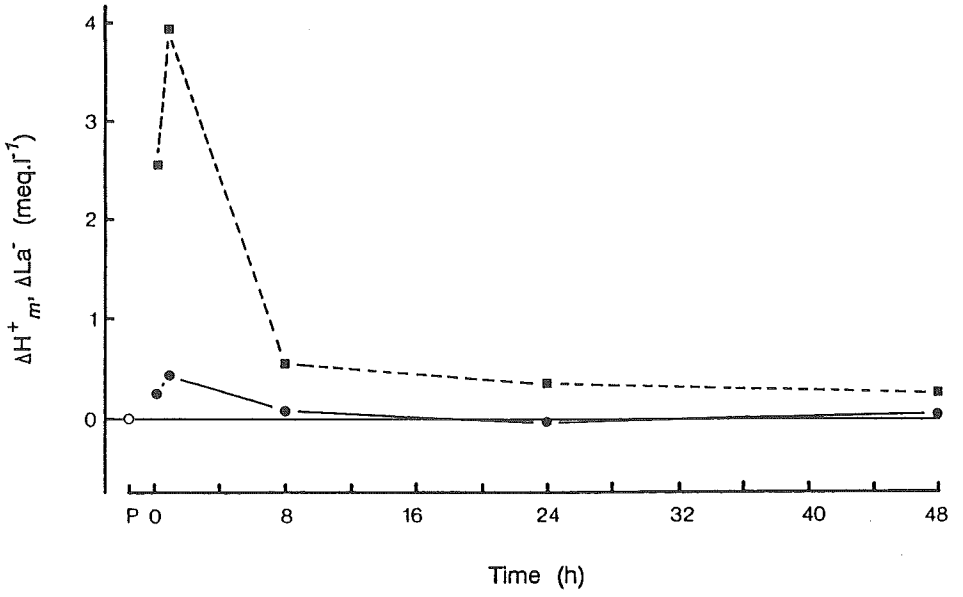

Fig. 3.12 Changes in the mean metabolic acid load (ΔH^+_m ■--■) and lactate load (ΔLa^- ●—●) after exercise at 17°C. By definition both are equal to 0 prior to exercise (P).

traditionally associated with the release of lactic acid is more properly recognised as originating from the hydrolysis of the ATP released during anaerobic metabolism, its breakdown producing ADP and H^+ ions (Portner et al., 1984; Zilva, 1978). Nevertheless, a 1:1 stoichiometry between lactate and metabolic hydrogen ions is expected (Hochachka and Mommsen, 1983). The discrepancy between the two is rather puzzling, especially since it creates a situation which is apparently detrimental to the animal (that is, a higher acid load in the haemolymph).

Several explanations for the discrepancy have been proposed including: 1) release of metabolically produced hydrogen ions originating from a source other than lactic acid; 2) removal of lactate from the bloodstream with a cation other than H^+ or 3) intracellular retention of lactate while the associated hydrogen ions are released into the bloodstream.

There is little information on the production of metabolic acids other than lactic acid by crustaceans. Available reports suggest that lactic acid is the main end-product of anaerobic respiration in crustaceans (Albert and Ellington, 1985; Gade, 1983).

It also seems improbable that lactate ions are released and then selectively removed from the haemolymph, although Jackson and Heisler (1982) found formation of calcium-lactate complexes in anoxic turtles. However, haemolymph [Ca^{2+}] remained constant throughout the post-exercise period (Fig. 3.10).

In relation to 3), empirical evidence for differential efflux rates of lactate and hydrogen ions is rather scarce, although Mainwood and Worsley-Brown (1975) and Benade and Heisler (1978) have both demonstrated higher efflux of hydrogen ions than lactate from isolated preparations of frog sartorius muscle. The reduced levels of bicarbonate found during the initial stages of recovery may have stimulated a preferential release of hydrogen ions in a similar

fashion to that described by Mainwood and Worsley-Brown (1975).

It is conceivable that lactate was retained within the muscles while hydrogen ions were released into the extracellular space, suggesting that regulation of tissue pH is more important than maintaining the acid-base stability of the haemolymph. In vertebrates pH_i is frequently observed to remain almost constant while pH_e decreases (Bennett, 1978). However, the release of H^+ into the haemolymph implies that more tissues than those producing the acid load will be affected by high levels of circulating hydrogen ions. While hydrogen ions may be preferentially released into the bloodstream the availability of a 'dumping site' in the form of external seawater suggests that excretion into the environment via ion exchange would be preferred to their internal retention, implying that there is a limit to H^+ excretion across the gills.

Anaerobic metabolism and the oxygen debt

Since there was only a small increase in the concentration of haemolymph lactate after exercise, it appears that *Jasus* relies predominantly on aerobic metabolism to support the workload required by this exercise protocol. However, $\dot{M}O_2$ was not restored to baseline levels until 8h after exercise. The oxygen debt estimated from Fig. 3.2 represents 3.5 mmol $O_2.kg^{-1}$. In vertebrate physiology the oxygen debt is usually partitioned into a rapidly repaid alactic portion and a more slowly repaid lactate portion (Bennett, 1978). However, the correlation between the change in lactate and the reduction of the oxygen debt was not close, since the total changes in lactate were very small, the highest lactate concentration being 0.5 $mmol.l^{-1}$. The energy derived from lactate release (in terms of O_2 equivalents) can be estimated from the lactate data, using the O_2 equivalence factor of 0.5 $mmol.l^{-1}$ O_2/$mmol.l^{-1}$ lactate (Wood and Randall, 1981a) and body

water content of 711 $ml.kg^{-1}$ determined from the data of Belman (1975) for *Panulirus interruptus*. This calculation assumes an even distribution of lactate throughout the intra- and extracellular spaces, and that these are the same as that measured in the haemolymph. From this calculation, the 0.5 $mmol.l^{-1}$ rise in lactate would constitute only 0.1 - 0.2 $mmol.kg^{-1}$ of the oxygen debt (in terms of O_2), or less than 5% of the total debt. If the changes occurring in ΔH^+_m are taken as being levels of lactate or other metabolites produced after exercise, then there is still a considerably discrepancy between this 'metabolite' component (1.4 $mmol.kg^{-1}$) and the total oxygen debt. A further possibilty is that not all lactate and H^+ ions are released into the haemolymph, and therefore the lactate portion of the O_2 debt has been underestimated. In *Leptograpsus variegatus* whole body [lactate] has been measured as 12 times higher than haemolymph [lactate] after exercise (P. Greenaway, pers. comm.). Nevertheless, recalculation based on a predicted whole body concentration using the total:haemolymph lactate ratio given above produces a value little different from that calculated from the changes in ΔH^+, and the lactate portion still constitutes only 50% of the oxygen debt. In *Jasus*, therefore, it seems that there is a large alactic portion - that is, energy is not only required to meet the elevated metabolic demands of the exercising tissues, but following exercise additional energy is required to replenish O_2 reserves and regenerate ATP.

Ammonia

The slight rise in haemolymph ammonia after exercise implies increased catabolism of proteins or amino acids, possibly for use as an additional metabolic energy source. While the significance of the ammonia changes in relation to acid-base balance are somewhat unclear, at physiological pH about 95% of ammonia is in the ionised

form (Mangum and Towle, 1977). If it is released as NH_3 then this would suggest that the acid load - ΔH^+_m - was even higher than that predicted by the analysis of the pH-bicarbonate diagram.

CHAPTER 4

CHANGES IN RESPIRATION AND ACID-BASE REGULATION DURING EMERSION.

ABSTRACT

When rock lobsters, *Jasus edwardsii*, were exposed to air for 8h, oxygen uptake decreased to less than half the aquatic level, scaphognathite frequency (f_{sc}) more than doubled and there was a transient bradycardia. With further emersion oxygen uptake increased slightly, but still remained below the pre-emersion levels, while heart rate rose above that recorded prior to air exposure. The persistently high scaphognathite rate was ineffective in flushing out CO_2, since the calculated value rose by 5 Torr over the 8h of emersion. Haemolymph lactate also increased by ~4 $mmol.l^{-1}$, indicating that there was a shortfall in oxygen supply which was met through anaerobic metabolism. Together these substances produced a mixed respiratory and metabolic acidosis, although the former was the main contributor to the fall in pH. The acidosis progressively deepened over the 8 hours' exposure to air. Haemolymph bicarbonate doubled, but the total change was small (about 3 $mmol.l^{-1}$). A small increase in haemolymph [Ca^{2+}] suggests that calcium carbonate release may be involved in acid-base regulation by raising the level of haemolymph buffers. Compensation, as evidenced by the rise in bicarbonate, was apparent only up to 4h emersion. Between 4 - 8h the concentration remained constant and pH fell faster than earlier in emersion. A pH-bicarbonate analysis revealed different release kinetics of lactate and metabolic hydrogen ions, which appeared to be due to the selective removal (or non-release) of the hydrogen ions produced with lactate.

Reimmersion produced rapid increases in $\dot{M}O_2$, f_{sc}, f_H and pH, while PCO_2 and lactate decreased. $\hat{\dot{M}}O_2$ and f_{sc} remained elevated over the first 24h of reimmersion, while it took 48h in water before heart rate returned to normal. Recovery of haemolymph pH was rapid and it had nearly been restored to the resting value by 4h. The initially rapid increase in pH was accompanied by an equally rapid fall in PCO_2 and lactate, although the rate of decrease of both these substances fell after 4h. Oxygen uptake remained higher longer than haemolymph [lactate], suggesting a large alactic portion to the oxygen debt. Compared to another subtidal crustacean, *Homarus gammarus*, *Jasus* appeared less able to adjust to air exposure. In both species the mechanisms controlling gas exchange were less effective for oxygen supply when in air, mainly because of the collapse of the gills in this medium. *Jasus*, however, had a lesser ability to regulate the acid-base status of its haemolymph than *Homarus*. It is suggested that this results from a lower maximum concentration of haemolymph bicarbonate, which limited the overall buffering power of the haemolymph in *Jasus*.

INTRODUCTION

As a sub-tidal animal, the chances of *Jasus edwardsii* becoming exposed to air are minimal under normal conditions. However, emersion of this species does occur during commercial handling. During capture, the animals are exposed to air, since in New Zealand rock lobsters are held dry on fishing vessels before being landed. *Jasus* is also known by the fishing industry to be able to survive several days exposure to air during live air freight, albeit at near-freezing temperatures since it is packed on ice for overseas shipment.

In aquatic crustaceans air exposure induces physical and

physiological changes. Most of the studies investigating air-breathing in aquatic crustaceans have examined species which are either periodically exposed to air, such as the intertidal crabs, or which leave their aquatic habitat temporarily when environmental oxygen levels become low or the temperature rises. Other studies, looking at mainly terrestrial species, have described the various physiological mechanisms permitting them to exploit their environment. Among the intertidal species which have been extensively studied are the shore crab *Carcinus maenas* (Truchot, 1975b; Taylor and Butler, 1978) and the red rock crab, *Cancer productus* (deFur and McMahon, 1984a, b). Taylor and his co-workers (Taylor and Wheatly, 1980, 1981; Taylor et al., 1987; Tyler-Jones and Taylor, 1988) have made an ongoing and detailed investigation of the changes imposed by air exposure on *Austropotamobius pallipes*, a species which voluntarily leaves water and which may be described as a facultative air-breather. Few studies have examined the exposure of sub-tidal species to air. Batterton and Cameron (1978) and deFur et al. (1988) gave some details of the changes occurring in *Callinectes sapidus* on emersion. It must be noted, however, that this species can be exposed to air at low tide (P. Greenaway, pers. comm.). Within the Macrura, Taylor and Whiteley (1989) examined changes in acid-base status of *Homarus gammarus* after 14h exposure to air, and Vermeer (1987) measured haemolymph variables and behavioural responses of the spiny lobster, *Panulirus argus*.

A major problem associated with the transition from water to air is the removal of the support for the gills normally provided by the medium. Exposure to air of water-breathers may induce collapse or coalescence of the gills. This would tend to reduce the available area for gas-exchange, to decrease diffusive conductance to oxygen and carbon dioxide exchange and probably would interfere with gill blood flow. In contrast, land-dwelling crustaceans often possess

highly modified and strengthened gill or lung-like structures, which permit effective gas exchange in air.

Branchial surfaces provide an important route for ion exchange in marine crustaceans (Kirschner, 1979). Thus, removal of aquatic animals from their normal habitat involves the loss of a major site for ion exchange and a potential site for acid-base exchanges.

The methods used to regulate haemolymph pH differ between aquatic and air-breathing crustaceans. In aquatic species there is a greater emphasis on ion exchange with sea water, whereas in air-breathing crustaceans alternative mechanisms such as intercompartmental ion exchanges are used to regulate pH. The ability of the ventilatory and circulatory systems to respond to a change in oxygen availability and to effectively excrete carbon dioxide, particularly if the gills coalesce, also determines how well a water-breathing species may adapt to air exposure. The ability of these animals to switch to alternative methods of regulating pH must therefore confer some advantage to survival in a foreign environment.

The experiments outlined in this chapter are concerned with the physiological responses of *Jasus* to air exposure, particularly in relation to the changes occurring in haemolymph acid-base balance. They examine circulatory and ventilatory changes and whether oxygen uptake is maintained in air. The capability to regulate haemolymph pH in the absence of branchial ion exchanges was also investigated.

MATERIALS AND METHODS

Animals of either sex and weighing between 300 and 670 gms were used in these experiments. They were held in the laboratory (temperature = 17 ± 1°C, salinity ~ 36‰) for several weeks before experiments were conducted, and were fed mussels until 1 week before

the commencement of experiments. The animals used had hardened carapaces and were judged to be in intermoult.

Experimental Protocol

In a fashion similar to that used in Chapter 3, three experimental series were used to maximise the accuracy of individual measurements. The experimental protocol was similar for each series. The animals were allowed to settle in water for at least 48h prior to emersion, permitting recovery from handling effects, operative procedures or other disturbances. The water was quickly siphoned from the animals (normally < 3min) without disturbing them. They were held in air for 8h, at the end of which time water was restored to the animals and recovery measurements were made. During the experiments the chambers were covered with black plastic sheeting to minimise disturbance. All experiments were carried out at 17 ± 1°C.

Series I: Oxygen Uptake Measurements

Forty eight hours before the experiments, the rock lobster was transferred to a watertight, Perspex respirometer (approximate volume = 4L) which was immersed in a waterbath thermostatted to 17 ± 1°C. Aerated water was circulated through the chamber when $\dot{M}O_2$ measurements were not being made. Measurements of aquatic oxygen uptake ($\dot{M}O_2$) were made by closing the chamber and measuring the drop in PO_2 within the chamber over a known time interval (usually around 8-12 minutes) with a PO_2 electrode (Strathkelvin, 1302) thermostatted to the same temperature as the waterbath. In resting animals the drop in PO_2 was between 7-15 Torr, although in the initial, post-emersion phase the change was greater. P_IO_2 never fell below 120 Torr. $\dot{M}O_2$ was calculated using Equation 2 given in Chapter 2, using the αO_2 value appropriate to the temperature used. Oxygen uptake during emersion

was measured using a method similar to that of Wood and Randall (1981b). The change in PO_2 in the respirometer was measured on discrete samples over 2h periods using the same PO_2 electrode. CO_2 was removed from the air by placing a small container of Carbabsorb within the respirometer. $\dot{M}O_2$ was calculated using the gas laws and the capacitance coefficient appropriate for gases in air (Dejours, 1981). Although the changes were small (around 2 Torr), calibration with air and with known mixtures of N_2 and air indicated a good reliability of the readings obtained using this method. A total of 4 consecutive measurements were made during the 8h emersion period, (plotted at mean times of 1, 3, 5 and 7h). In both aqautic and aerial measurements control respirometers were run to detect changes in PO_2 resulting from micro-organisms. Any background change in PO_2 was subtracted from the changes in PO_2 caused by the lobsters.

Series II: Scaphognathite and Heart Rate Recordings

Total scaphognathite rate (f_{sc}, the sum of the left and right frequencies) and heart rate (f_H) were measured using the impedance technique described in Chapter 2. Fine, plastic-coated, silver wires, bared for about 3-4 mm at the tip, were inserted through small holes drilled on either side of the scaphognathites or heart and were secured with hot glue. The impedance changes were amplified by Strathkelvin Impedance Coupler units (Biosciences A100 power supply) and recorded on a Gould 2202 recorder.

Series III: Haemolymph Acid-Base Measurements

Changes in pre-branchial acid-base characteristics were measured on a total of 20 animals. Each data point is the mean of 7-8 animals. Each animal was exposed to air and then sampled at only one time interval during emersion and recovery. The animal was then left undisturbed for 48h before it was re-emersed and a further sample

removed. Pre-branchial pH, total CO_2 (C_{CO2}), lactate, calcium and osmotic pressure were subsampled from approximately 0.7 - 1.0 ml of haemolymph removed anaerobically from the infrabranchial sinus at the base of the walking legs.

Analyses were performed as described previously (Chapter 3). Briefly, pH was measured on 70 μl samples with a Radiometer microelectrode (G297/G2), thermostatted to 17 ± 1°C and calibrated with Radiometer precision buffers. Total CO_2 was measured on 20 μl haemolymph samples with the Cameron method as described in Chapter 3. Haemolymph lactate was determined on either 50 or 150 μl subsamples with a Boehringer food analysis kit for lactate, modified and calibrated as detailed in Chapter 3. Haemolymph calcium was determined on 10 μl samples using a Varian Techtron 1200 atomic absorption spectrophotometer. Pre-branchial osmotic pressures were measured with a Wescor 5100C vapour pressure osmometer. Non-bicarbonate buffer lines were determined *in vitro* on the haemolymph of 6 animals using the method described in Chapter 3.

Calculations

Calculations of PCO_2, bicarbonate ($[HCO_3^- + CO_3^{2-}]$), the change in metabolic acid load ($[\Delta H^+_m]$), the lactate load ($[\Delta La^-]$) and the slope of the non-bicarbonate buffer line (β) are given in Chapter 3.

Statistical treatment

All data are given as means ± 1 S.E.M. Statistical analysis was performed with a GLM ANOVA, and statistical differences between means compared using Fisher's Least Significant Difference test. Significance was designated at $p<0.05$.

RESULTS

Survival

Three groups were used to study emersion in *Jasus*, those used to study the influence of emersion on oxygen uptake, those for ventilatory and circulatory measurements, and those whose acid-base response was examined. Of the animals sampled at each point during one emersion and reimmersion trial (the animals used for oxygen uptake (Series I) and ventilation and circulation measurements (Series II)) relatively few died from the effects of emersion. Only 4 of the 22 animals used in Series I and II did not survive air exposure (~18%). Data was rejected from any animal that died during a trial. Those animals which died were previously sluggish and had shown little response to tactile stimulation, even of the antennae. Higher mortality was recorded in the animals tested for acid-base characteristics (6 dead from a total of 20 animals). The lower survivorship in this group presumably reflected the effects of haemolymph sampling, rather than solely emersion. In contrast to the other two experimental series, some of the animals used to investigate acid-base changes also died during reimmersion, additionally pointing to more stressful conditions for these animals.

Respiratory Variables

The rates of oxygen consumption ($\dot{M}O_2$), total ventilation frequency (f_{sc}) and heart rate (f_H) at rest, during aerial exposure of 8h and during the 48h of recovery in water following emersion are shown in Figs. 4.1-4.3.

Figure 4.1 illustrates the changes in $\dot{M}O_2$ during emersion and reimmersion. The histogram arrangement of the data during the air exposure period reflects the 2-hourly intervals over which samples were taken, while the symbols represent essentially instantaneous

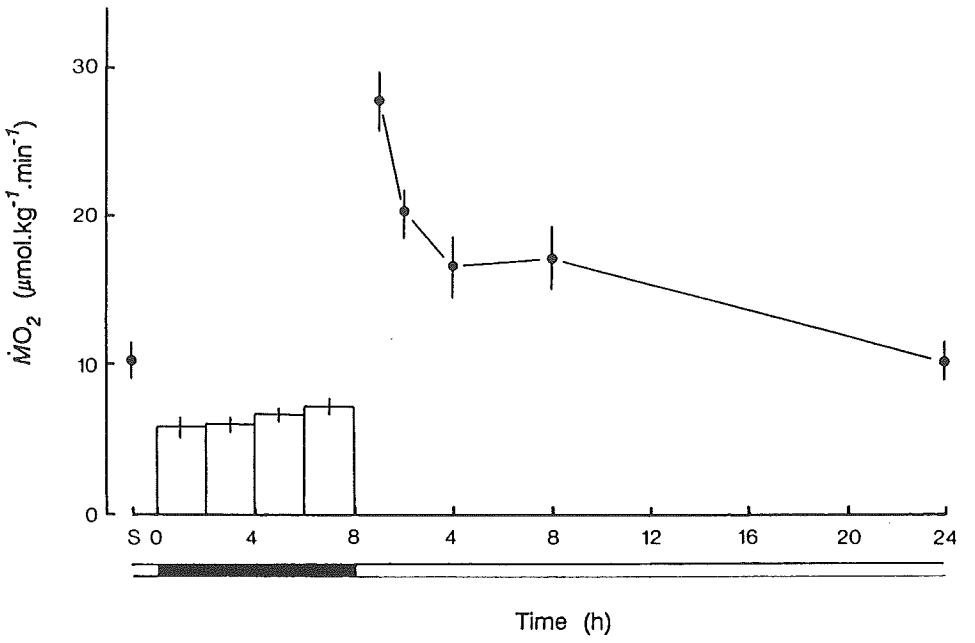

Fig. 4.1 The effect of 8h air exposure, followed by 24h reimmersion, on oxygen uptake ($\dot{M}O_2$) in *Jasus edwardsii* at 17°C. The horizontal bar beneath the time base indicates time in water (unshaded) and time in air (shaded). Initial resting values (S) were determined after 48h in water. The histogram arrangement of the data during air exposure represents mean oxygen uptake determined over 2h intervals. Data = mean ± 1 SEM, n = 10.

(approximately 10 min.) values. $\dot{M}O_2$ in rock lobsters settled for 48h before emersion was 10.27 ± 1.22 (10) μmol.kg^{-1}.min^{-1}, and is similar to the settled values reported for this species in Chapters 2 and 3 (no significant difference, $p>0.05$). In each of the animals tested, $\dot{M}O_2$ fell when the animals were first exposed to air. Mean $\dot{M}O_2$ was only 56% of the pre-emersion value at the mean time of 1h (significant, $p<0.05$). While oxygen uptake rose with further emersion, by 4-6h there was no significant difference from the control value.

By 1h of reimmersion, the rate of oxygen consumption increased dramatically to more than 2.5 times the resting, pre-emersion rate (significant, $p<0.001$), and was nearly 4 times the final mean value recorded in air. Following the 1h peak the rate of oxygen consumption was gradually reduced. Full restoration back to the pre-emersion level of oxygen usage was relatively protracted. After 8h reimmersion $\dot{M}O_2$ measured 17.26 ± 2.18 μmol O_2.kg^{-1}.min^{-1}, which was still significantly higher than that measured pre-emersion ($p<0.001$). Mean $\dot{M}O_2$ at 24h was 10.35 ± 1.32 μmol.kg^{-1}.min^{-1}, and was not significantly different from the pre-emersion rate.

The mean, total scaphognathite rate (f_{sc}) at rest was 142.5 ± 20 bpm (Fig. 4.2). Three minutes of emersion produced a highly significant ($p<0.001$) doubling in ventilation frequency. With only small variations in the mean values, f_{sc} was maintained at an almost constant rate of 270 bpm during air exposure and was significantly higher than resting f_{sc} at all times ($p<0.001$).

Scaphognathite beat frequency increased still further on reimmersion, rising by an additional 30 bpm after only 3 minutes in water. The peak rate was seen at 1h, where mean f_{sc} was determined as 340.4 ± 10.0 bpm. At 2h it had fallen by only 8 bpm from the 1h rate, but between 2 and 8h ventilation frequency decreased quite steadily. Scaphognathite rate at 8h was still significantly higher than the

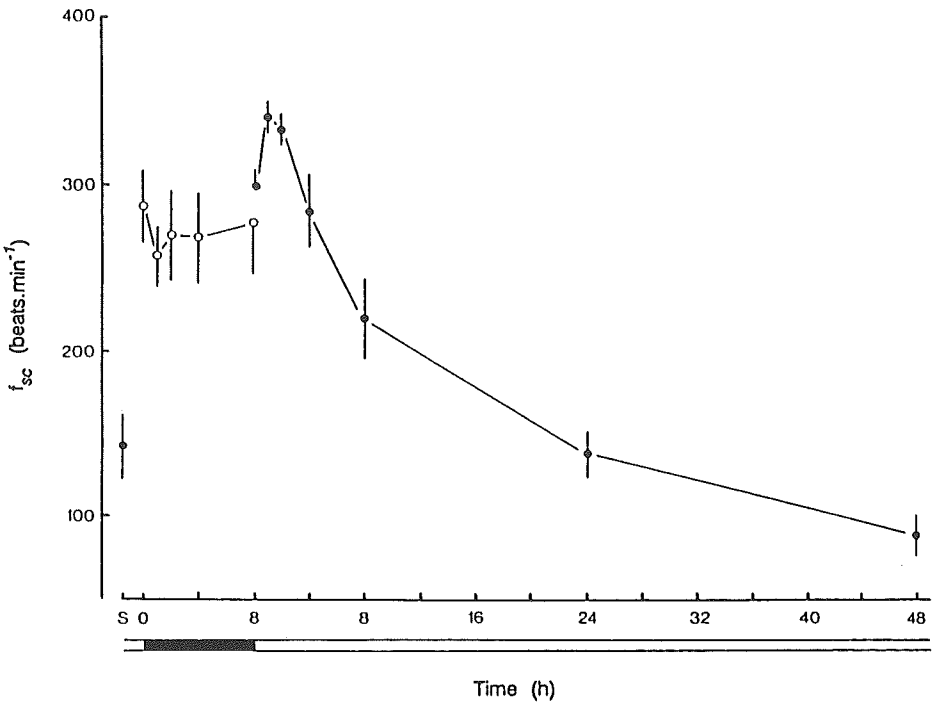

Fig. 4.2 Mean change in scaphognathite frequency (f_{sc} = sum of the left and right scaphognathites) during emersion and subsequent reimmersion at 17°C. Open symbols reflect changes during 8h air exposure, and closed symbols refer to measurements made on immersed animals. Data are given as mean ± 1 SEM, n = 8. The shaded area of the horizontal bar below the time base indicates the emersion period.

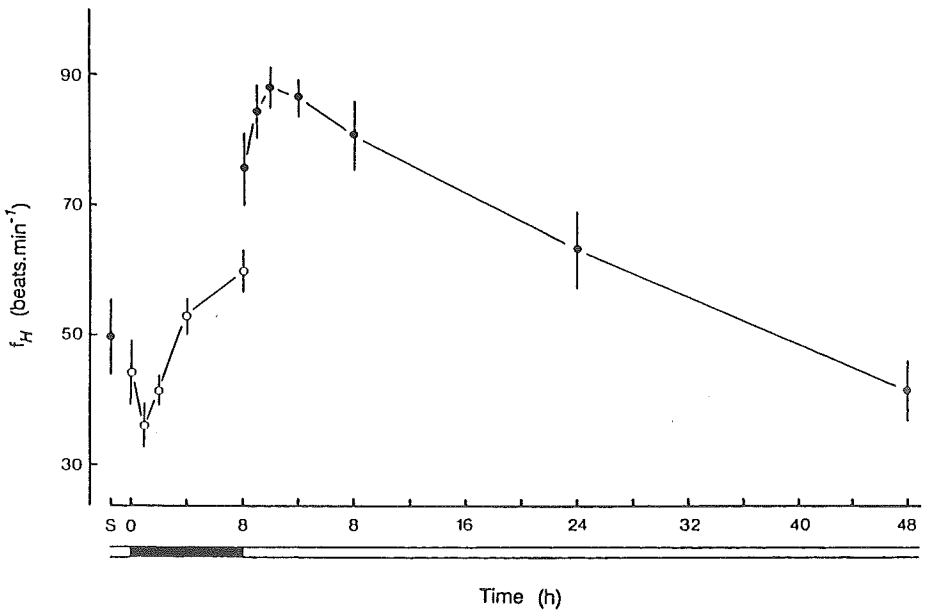

Fig. 4.3 The effect of emersion and subsequent reimmersion on heart rate (f_H) in *Jasus edwardsii* at 17°C. n = 8. Other details as in Fig. 4.2.

value recorded pre-emersion ($p<0.02$). Even though there was no significant difference between the control and 24h values, ventilation frequency continued to decrease with further recovery; 48h in water produced an f_{sc} which was statistically lower than that recorded prior to emersion ($p<0.05$).

The pattern seen in heart rate during emersion and during the subsequent recovery in water is shown in Fig. 4.3. From the pre-emersion rate of 49 ± 5.9 bpm, a slight bradycardia had developed within 3 minutes' exposure to air, and after 1h f_H had decreased further to 36.1 ± 3.4 bpm, a significant depression compared to f_H at rest ($p<0.02$). By 2h into emersion f_H had started to rise again. This continued throughout the remainder of the air-exposure period, f_H reaching a final, emersed value of 59.8 ± 3.4 bpm (not significantly different from control value). Reimmersion produced a rapid and significant tachycardia, f_H at 3 minutes having increased over the 8h emersed value by about 25 bpm ($p<0.001$). The highest heart rate occurred slightly later than those of either $\dot{M}O_2$ or f_{sc}, the peak value being reached after 2h in water as compared to 1h for both $\dot{M}O_2$ and f_{sc}. The rate decreased the longer the animals were in water, but even at 24 hours reimmersion f_H was still significantly higher than the value measured in water prior to air exposure ($p<0.05$).

Acid-Base Variables

Changes in pH, calculated PCO_2, bicarbonate ($[HCO_3^- + CO_3^{2-}]$) and lactate measured before and during 8h emersion and during 24h reimmersion are shown in Figs. 4.4-4.7.

At rest, mean pre-branchial pH was 7.669 ± 0.019 (8) units. The haemolymph became progressively more acidic over the eight hours (Fig. 4.4). and by 8h pre-branchial pH had fallen to 7.343 ± 0.031 (8), an almost 2-fold increase in hydrogen ion concentration ($p<0.001$). Haemolymph pH increased rapidly on return to water. At 1h

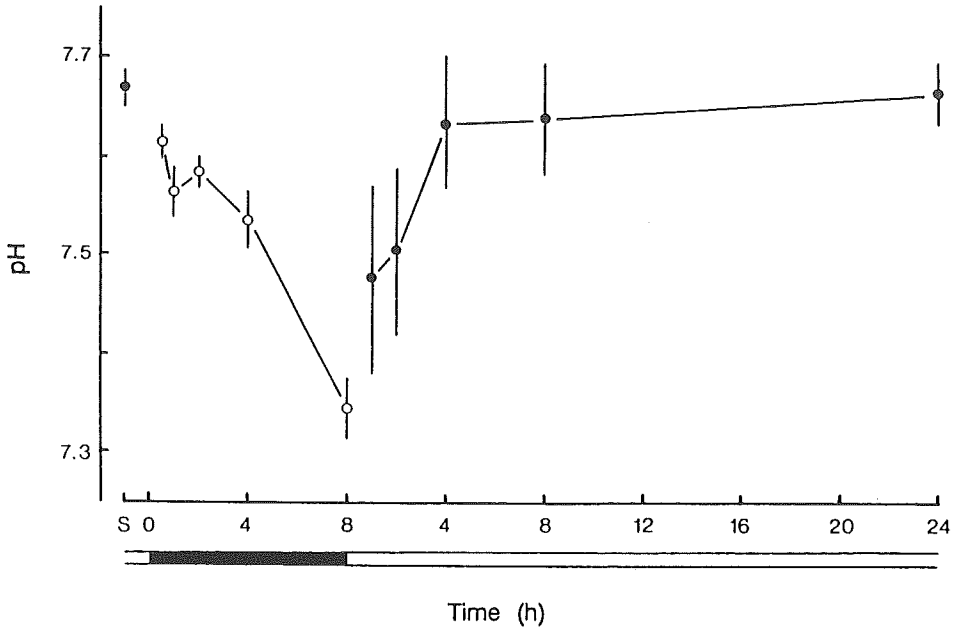

Fig. 4.4 Changes in pre-branchial haemolymph pH during emersion (open symbols) and reimmersion (closed symbols) at 17°C. n = 7-8. Other details as described in Fig. 4.2.

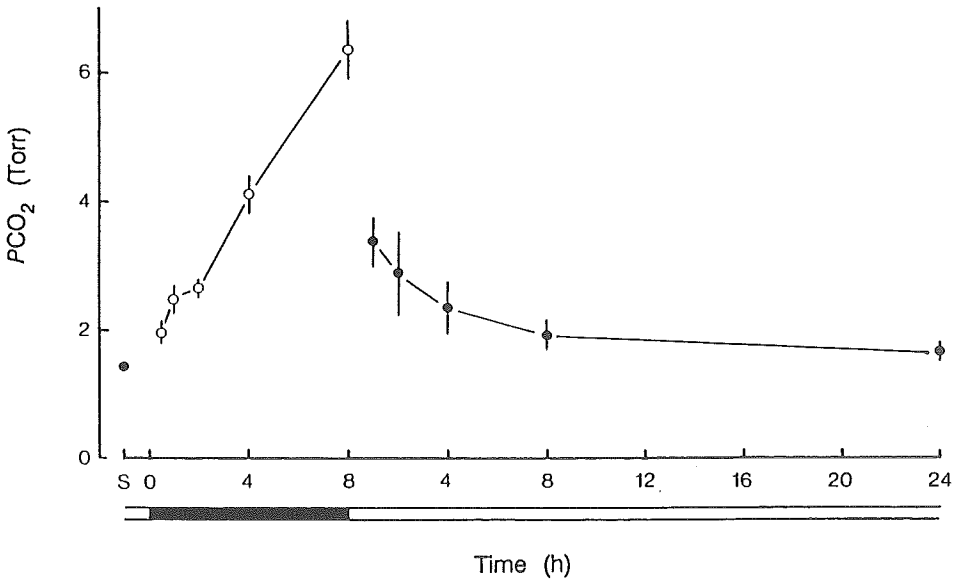

Fig. 4.5 Changes in calculated PCO_2 during 8h emersion and subsequent reimmersion in *Jasus edwardsii*. n = 7-8. Other details as in Fig. 4.2. Where no error bars are shown they lie within the limits of the symbol.

it had risen 0.14 units from the 8h emersed value, but after 4h pH was restored to a value not significantly different to that measured pre-emersion.

Calculated PCO_2 increased steadily over the 8h period in air, and was significantly elevated after 1h ($p<0.05$). The CO_2 tension in the haemolymph increased more than 4-fold during the emersion period, peaking at around 6.6 Torr at 8 hours (Fig. 4.5). There was an initially rapid drop in PCO_2 on reimmersion. Within the first hour in water there almost a 70% decrease in PCO_2. While it continued to fall, it still took 8 hours in water for the CO_2 tension of the haemolymph to be restored back to a level not significantly different from that at rest.

The calculated bicarbonate concentration ($[HCO_3^- + CO_3^{2-}]$) also rose during air exposure, and was significant higher than the pre-enersion concentration after 1h ($p<0.05$, Fig. 4.6). It continued increasing with emersion, rising to a plateau of around 6.6 $mmol.l^{-1}$ between 4h and 8h. This process was reversed when the animals were returned to water, $[HCO_3^- + CO_3^{2-}]$ decreasing within an hour. The concentration was still significantly elevated over the pre-emersed level at 4h, and it was not until 8h that there was no significant difference to the resting concentration.

The haemolymph lactate changes during exposure to air and during subsequent reimmersion are shown in Fig. 4.7. At rest there was a very low concentration of lactate in the haemolymph (<0.1 $mmol.l^{-1}$), but during emersion this increased considerably, rising to a final concentration of 4.21 ± 0.77 (8) $mmol.l^{-1}$ at 8h. The degree to which individual animals responded to aerial exposure by the production of lactate varied considerably, ranging from 1.0 to 7.7 $mmol.l^{-1}$ at 8h. The rate of increase in haemolymph [lactate] was greatest between 4 and 8h. Nearly two-thirds of the lactate appeared during this interval. As observed for the other acid-base variables, reimmersion

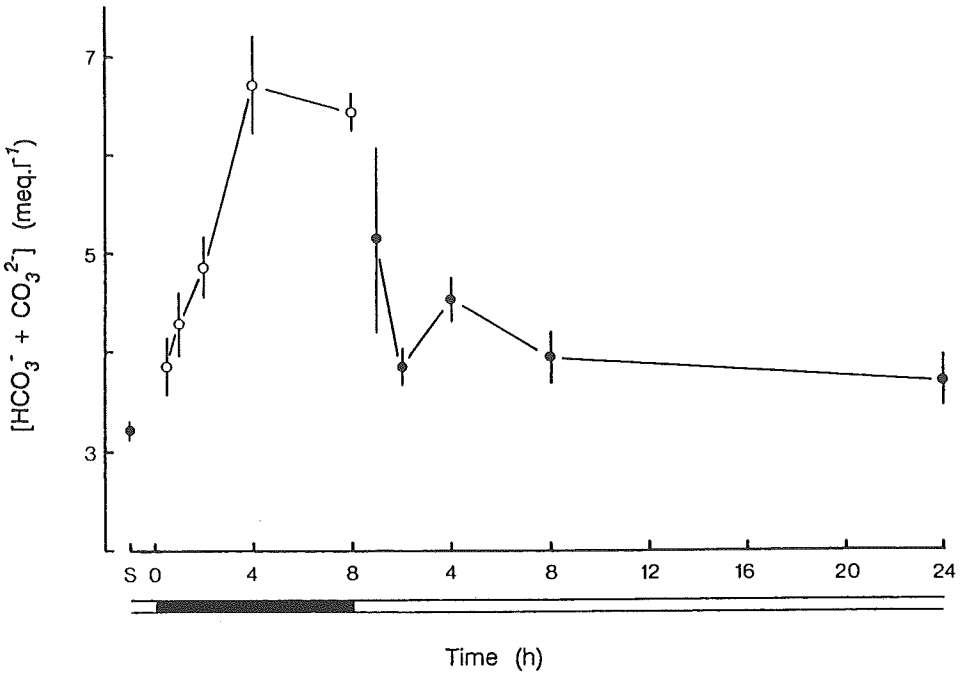

Fig. 4.6 The effect of emersion and subsequent reimmersion on bicarbonate concentration ($[HCO_3^- + CO_3^{2-}]$) in pre-branchial haemolymph of *Jasus edwardsii* at 17°C. n = 7-8. Other details as in Fig. 4.2.

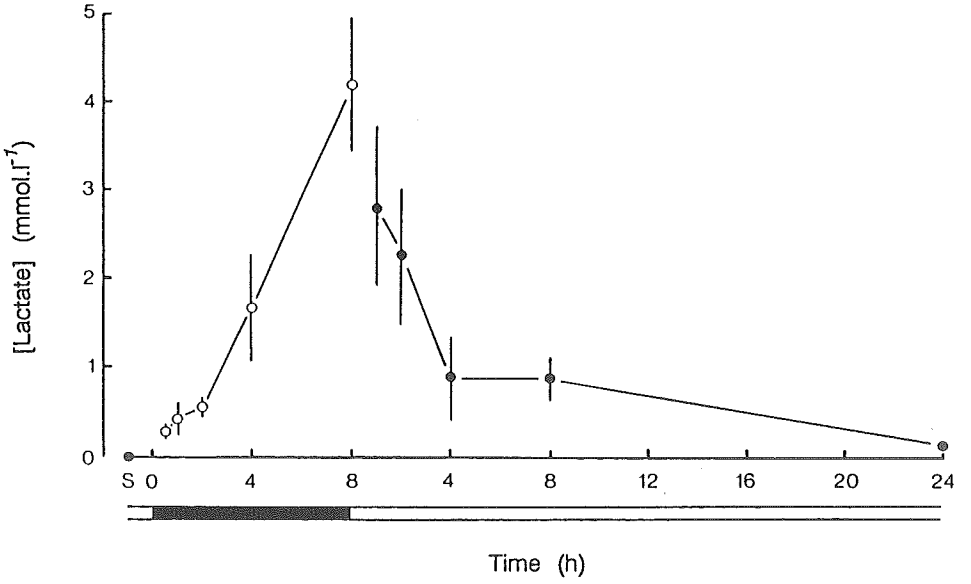

Fig. 4.7 Changes in haemolymph [lactate] during 8h emersion and 24h reimmersion at 17°C. n = 7-8. Other details as described in Fig. 4.2. Where error bars are not shown, they lie within the symbol.

resulted in a reversal of the changes occurring during emersion. [Lactate] fell by 2 $mmol.l^{-1}$ when the animals had been in water for 2h, and by 4-8h the concentration had decreased an additional 1 $mmol.l^{-1}$. However, even after recovery of 8h the concentration was still significantly elevated over the concentration measured prior to emersion ($p<0.05$). At 24h mean [lactate] was 0.14 ± 0.05 $mmol.l^{-1}$, which was similar to the initial, submerged value.

Acid-Base Analysis

Fig. 4.8 presents a summary of the pH, $[HCO_3^- + CO_3^{2-}]$ and PCO_2 changes occurring in the haemolymph of *Jasus* during emersion and recovery. Also shown on the pH-bicarbonate diagram is the non-bicarbonate buffer line determined *in vitro*, which had a mean slope of -8.93 $meq.l^{-1}.pH\ unit^{-1}$. Over the first hour in air pH and bicarbonate followed the buffer line, suggesting that the acidosis was caused solely by respiratory (CO_2) acids. This is apparently at variance with the lactate data presented in Fig. 4.7, which showed that a small increase in [lactate] had developed by that time. Between 1 and 4h emersion haemolymph pH remained relatively constant in spite of further increases in PCO_2 and [lactate] (Figs. 4.5 and 4.7). This stability in pH was associated with a further increase in $[HCO_3^- + CO_3^{2-}]$ which rose above the buffer line. No further compensation occurred between 4 and 8h emersion, and there was a large drop in pH over this time.

During the first hour of reimmersion haemolymph pH increased through a respiratory alkalosis. At 2h recovery there was a metabolic acidosis relative to 1h, indicating a loss of bicarbonate from the system. This was later recovered and by 24h the decrease in both respiratory and metabolic acids was sufficient to restore pH back to the pre-emersion value.

Fig. 4.9 shows the change in metabolic acid load (ΔH^+_m) against

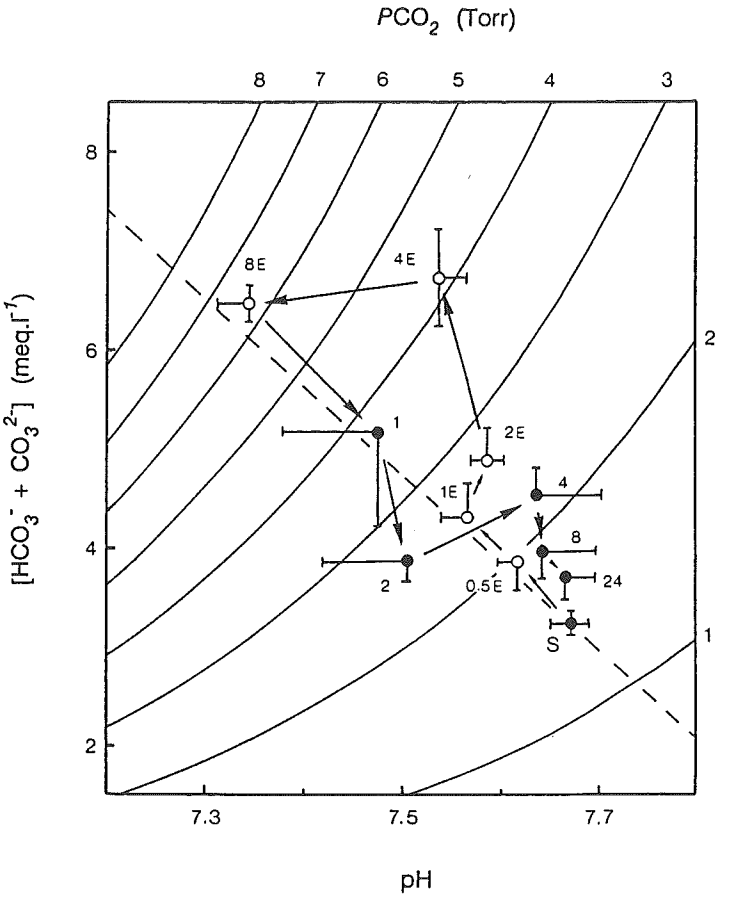

Fig. 4.8 pH-bicarbonate diagram illustrating the effect of emersion (○) and reimmersion (●) on pH, $[HCO_3^- + CO_3^{2-}]$ and calculated PCO_2. Sample times are given beside each point, those with an 'E' referring to measurements made in the emersion period. 'S' = settled value. The dashed line (– – –) represents the non-bicarbonate buffer line determined *in vitro*. Temperature = 17°C.

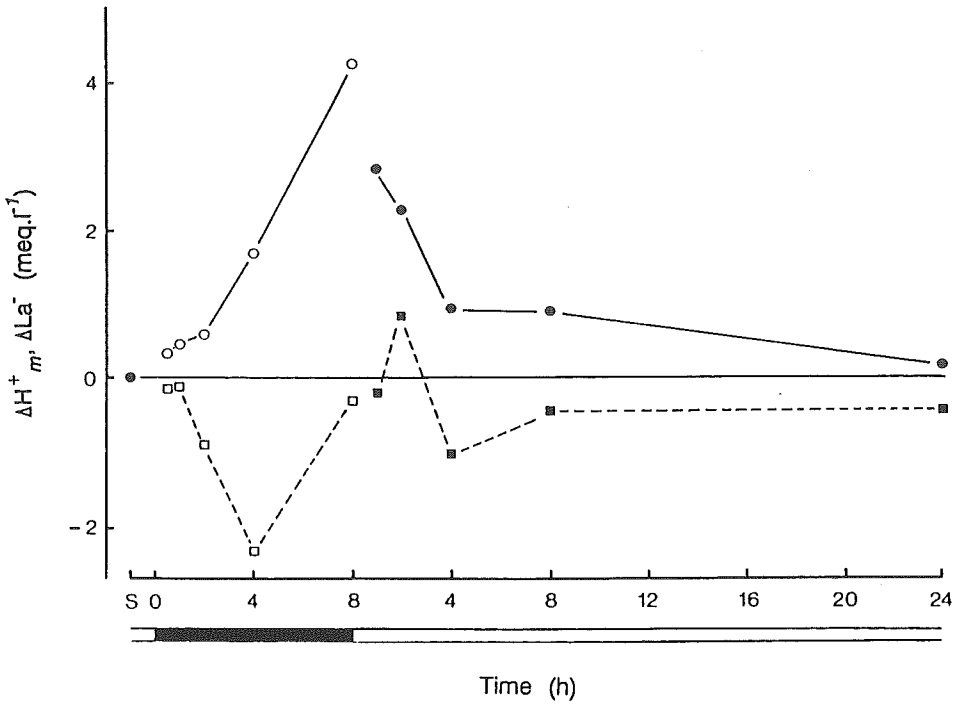

Fig. 4.9 Mean changes in the calculated metabolic acid load (ΔH^+_m □■ – –) and lactate load (ΔLa^- ○● ——) during emersion and subsequent reimmersion. By definition, the value of both variables is equal to zero in settled, submerged animals (S). Other details as described in Fig. 4.2.

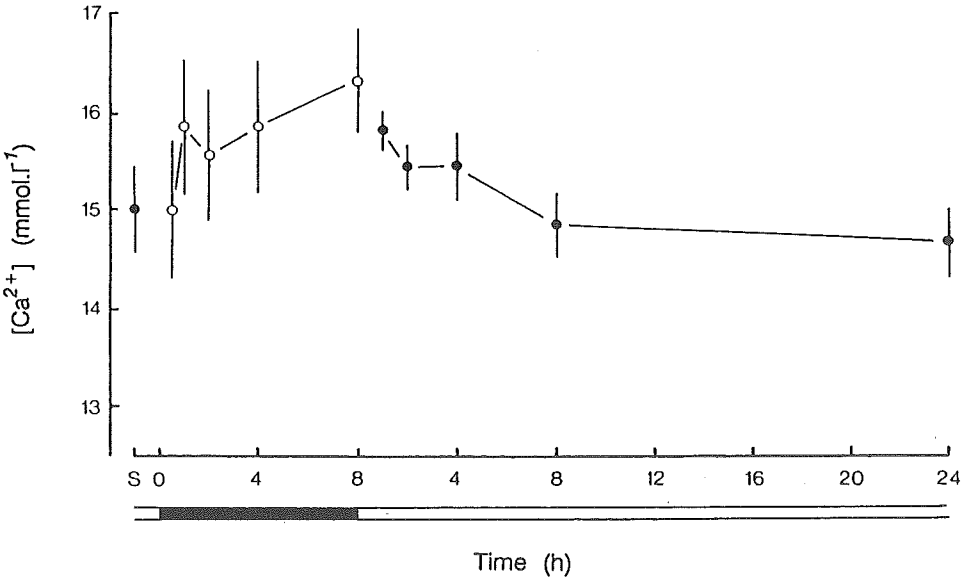

Fig. 4.10 Changes in the mean concentration of calcium in pre-branchial haemolymph during 8h emersion and 24h reimmersion. n = 7-8. Other details as in Fig. 4.2.

that of lactate (ΔLa^-). By definition, in settled animals both variables are equal to zero. There were marked differences in magnitude and sign of the two variables throughout emersion and recovery. ΔLa^- rose consistently during air exposure, whereas ΔH^+_m was always lower than the resting value (indicating a base excess) and up to 4h steadily decreased, before approaching zero at 8h emersion. During reimmersion ΔLa^- fell towards zero, while ΔH^+_m remained relatively constant at around -0.5 meq.l^{-1}.

Calcium

Changes in the concentration of calcium during aerial exposure and recovery from emersion are illustrated in Fig. 4.10. Over the 8h in air mean [Ca^{2+}] rose from 15.00 ± 0.45 mmol.l^{-1} at rest to 16.32 ±0.53 mmol.l^{-1} at the end of the exposure period ($p<0.05$). During reimmersion this effect was reversed, with none of the other changes in calcium significantly different from the control concentration.

The measured changes in osmotic pressure were also small. There was a slight (<1%) increase over the 8h air exposure (1027 mOsm.kg^{-1} in settled, submerged animals, 1036 mOsm.kg^{-1} at 8h emersion), but the change was not statistically significant ($p>0.1$).

DISCUSSION

When *Jasus* was exposed to air mean oxygen consumption was lower than in water. $\dot{M}O_2$ after 1h was just over half the resting $\dot{M}O_2$, and despite a partial recovery with further emersion, at 7h it was still only 70% of the initial value. Such a reduction in oxygen uptake implies that either *Jasus* reduces its overall metabolic rate in air and therefore the requirement for oxygen, or the animals retain their normal metabolic rate, but there is a shift towards an increased

reliance on anaerobic mechanisms, or both. The lactate data given in Fig. 4.7 indicate that the animals' metabolic requirements were not able to be met solely through aerobic metabolism, since lactate rose continuously throughout the emersion period. The energy produced via anaerobic metabolism can be estimated in terms of oxygen equivalents, using the change in [lactate] (Wood and Randall, 1981a). This assumes that the intracellular and extracellular lactate concentrations are the same and that these are equal to haemolymph [lactate]. Using the total body water content of 711 $ml.kg^{-1}$ calculated from the data of Belman (1975) for *Panulirus interruptus*, anaerobic metabolism produced energy equivalent to about 1.5 $\mu mol\ O_2.kg^{-1}.min^{-1}$. Adding the lactate equivalent changes to the measured values of oxygen consumption suggests that during the first few hours of emersion the metabolic rate was reduced over the normal rate in water, but by 8h it had risen to approximately the same as that measured pre-emersion.

Reduced levels of oxygen uptake have been documented for other crustacean species during emersion, including the crabs *Liocarcinus puber* (aerial $\dot{M}O_2$ = 19% of aquatic $\dot{M}O_2$, Johnson and Uglow, 1985), *Cancer productus* (~ 10 - 20%, deFur and McMahon, 1984a), *Pachygrapsus crassipes* and *Eurytium albidigitum* (45% and 5%, respectively, Burnett and McMahon, 1987), *Callinectes sapidus* (35%, O'Mahoney and Full, 1984) and the lobster *Homarus vulgaris* (= *gammarus*) (14%, Thomas, 1954). *Jasus* conforms to the general pattern shown by most other aquatic crustaceans of a reduction in oxygen uptake during emersion.

There are, however, a few examples of aquatic animals sustaining or even increasing oxygen uptake during emersion, most notably *Austropotamobius pallipes* (Taylor and Wheatly, 1980, 1981) and *Carcinus maenas* (Taylor and Butler, 1978). Both authors described physiological adaptations permitting an increase in oxygen uptake, and Taylor and Butler (1978) additionally suggested that the

lamellate gills of *Carcinus* may be more resistant to collapse in air because of the rigidity of the chitinous covering of the gills. This latter advantage may not be available to the macrurans, since the latter authors measured a blood-water barrier 6 μm thick in *Carcinus*, of which 5 μm was chitin, whereas Rogers (1982) reported a total diffusion barrier (epithelial cells and chitin) of 2.8 - 4.2 μm in *Jasus novaehollandiae*, a species closely related to *J. edwardsii*.

It is interesting that, even though oxygen consumption was reduced during emersion, scaphognathite beat frequency approximately doubled within 3 minutes exposure to air. However, it is not clear what factor was responsible for initiating the increase in ventilation frequency. McMahon and Wilkens (1975) argue that the hypoxic response of the lobster is mediated via direct reception of oxygen levels by the pacemaker systems controlling scaphognathite rate, and Massabuau et al. (1980) have directed similar arguments to the control of ventilation in the crayfish *Austropotamobius* (= *Astacus*) *pallipes*. It is possible that the increase in f_{sc} observed in *Jasus* also resulted from an internal hypoxia. Nevertheless, the rapidity of the response suggests that some other factor, perhaps the detection of the physical loss of water, may be at least partially involved in the initial change in scaphognathite rate.

In animals that usually never encounter air, scaphognathite beating frequencies have increased quite dramatically on emersion, in some cases rising more than 4-fold (e.g. large *Cancer productus*, deFur and McMahon, 1984a). By contrast, in facultative air-breathers the ventilation rate may increase only slightly on emersion (e.g *Austropotamobius pallipes*, Taylor and Wheatly, 1980, 1981; *Orconectes rusticus*, McMahon and Wilkes, 1983) or, in some cases such as small, high-shore *Cancer productus*, aerial f_{sc} may even be slightly depressed compared to the aquatic rate (deFur and McMahon, 1984a).

However, deFur et al. (1988) found a similar reduction in f_{sc} in small, subtidal *Callinectes*, and suggested that this response is ultimately related to differences in morphometry between large and small individuals. Nevertheless, a reduction in scaphognathite activity implies that the metabolic costs associated with ventilation will be lower in species showing this response than in those, such as *Jasus*, which increase f_{sc}.

In *Jasus* and other aquatic animals a portion of the oxygen consumed during emersion is probably expended on pumping air to respiratory surfaces across which there can be little gas exchange. Coalescence of the gills through the effect of surface tension forces and the lack of a buoyant medium would be expected to increase the diffusion distance for gas exchange. In addition, there are a number of reports (e.g. Batteron and Cameron, 1978; Taylor and Wheatly, 1980; O'Mahoney and Full, 1984) of a large reduction in ventilation volume in air, despite stable or elevated scaphognathite rates, implying a reduction in scaphognathite stroke volume in this medium ($\dot{V}_g$ equalled 4% of $\dot{V}_w$ in *Austropotamobius*, Taylor and Wheatly, 1980). Thus the increase in f_{sc}, when coupled with coalescence of the gills and probably a reduction of ventilation volume, appears energetically costly and mal-adaptive for respiration in air.

Coalescence of the gills presumably impairs both O_2 and CO_2 transfer. Emersion resulted in a steady rise in the level of carbon dioxide, even at a time when, because of the lower level of oxygen uptake, it might be expected to remain steady or decrease, implying differential conductance across the gills for CO_2 and O_2. The increased rate of beating of the scaphognathites was apparently ineffective in reducing the CO_2 tension of the haemolymph since emersion produced a 4-fold increase in PCO_2. The absolute change in PCO_2 in *Jasus* is similar to those reported for *Homarus* after 14h exposure to air (Taylor and Whiteley, 1989), for *Carcinus maenas* (9h,

Truchot, 1975b), *Austropotamobius pallipes* (Taylor and Wheatly, 1981), and the 4h emersed values are similar to those over the same time interval in *Cancer productus* (deFur and McMahon, 1984b). High CO_2 levels have also been found in fish during emersion (e.g. *Channa argus*, Ishimatsu and Itazawa, 1983; *Anguilla rostrata*, Hyde et al., 1987). In many of these aquatic species the rise in PCO_2 during emersion probably does not reflect a consequence of hypoventilation in the manner usually associated with air breathers. Even though they normally experience a reduction in ventilation volume in air, the respiratory frequency generally rises.

Cardiac activity was also affected by emersion, over the first hour heart rate falling to about two-thirds of the aquatic rate. Bradycardia on emersion is a common feature in fish (Satchell, 1971), in some cases f_H being reduced to 10% of the pre-emersion rate (e.g. Garey, 1962). Similar responses have been reported for adult *Carcinus maenas* (Depledge, 1984) and small *Cancer productus* (deFur and McMahon, 1984a) on introduction to air, the latter authors also reporting a large decrease in cardiac output, both stroke volume and rate apparently being reduced. A possible factor in reducing f_H may be the probable change in blood oxygen levels in air. An additional or alternative factor mediating the reflex bradycardia may be the clumping of the gills in air, acting via (1) changes in internal gill pressure detected by baroreceptors such as those proposed for trout by Wood and Shelton (1980), or (2) tactile reception, perhaps of changes in relative gill position, by mechanoreceptors located in the branchial chamber, which Field and Larimer (1975) found caused a reflex bradycardia in the crayfish *Procambarus clarkii*.

Between 1 and 2h f_H started to rise. It is possible that this results from drying of the gills, which might increase the available surface area for gas exchange. Although drying might at first cause external surface tension pressures to rise, eventually almost

complete drying of the branchial filaments would reduce the external pressure on the gills, permitting resumption of blood flow.

The continuous rise in lactate throughout emersion suggests a shortfall in oxygen supply. The rate of production or appearance of lactate in the haemolymph was somewhat variable, the lowest rate occurring between 1-2h, and increasing thereafter to a maximum between 4-8h emersion. Since $\dot{M}O_2$ was actually increasing over this time, an increase in the rate of production of lactate is initially surprising. However, lactate may not be released immediately. The rate of appearance of lactate in the haemolymph may depend on accumulation to high concentrations intracellularly. If the changes in lactate are compared to the pattern observed in f_H there is a general correlation between the rate of appearance of lactate and heart rate. If cardiac output generally parallels heart rate, the slower initial rate of lactate appearance during the first hour of emersion might then reflect reduced perfusion of the lactate-producing tissues. This ability to retard release of lactate may confer some physiological advantage to the animal under conditions when high production of lactate occurs. This may result from the influence of lactate on haemocyanin oxygen affinity, which is known to increase under high lactate concentrations (Truchot, 1980; Bouchet and Truchot, 1985). The more rapid release of lactate may then enhance oxygen uptake across the gills during the latter part of emersion.

The limitations of gas exchange, resulting in the rise in PCO_2 and the increase in anaerobic metabolism as shown by the rise in haemolymph lactate, produced a mixed respiratory and metabolic acidosis, so that at the end of the 8 hours hydrogen ions had doubled in concentration.

The acid-base response to air exposure can be classified into different categories. There are some species, including *Cyclograpsus*

lavauxi (Innes et al., 1986), *Hemigrapsus nudus* and *Pachygrapsus crassipes* (Burnett and McMahon, 1987) which show excellent compensation for acid-base disturbances resulting from emersion. This response is characterized by the mobilisation of internal buffers which counteract the internal hypercapnia and the increase in metabolic acids which initially act to depress pH. Long-term emersion produced only a slight haemolymph acidosis in *Austropotamobius* and *Carcinus* (Taylor and Wheatly, 1980, 1981; Truchot, 1975b). An intermediate emersion response is shown by species such as *Homarus*: although haemolymph pH may fall during emersion, it does not fall continuously, and usually reaches a plateau. Buffering usually increases to a lesser degree in these species than in others better adapted to air exposure (Taylor and Whiteley, 1989). The third group consists of species which show little ability to raise the buffering power of the haemolymph, resulting in the haemolymph becoming progressively more acidic (e.g. *Eurytium albidigitum*, (Burnett and McMahon, 1987).

Jasus shows only a limited ability to regulate its haemolymph pH when it is exposed to air. Taylor and Whiteley (1989) found that after 14h aerial exposure, haemolymph pH in *Homarus* was regulated to that found after emersion of 3h. By contrast, haemolymph pH in *Jasus* fell throughout emersion and most rapidly between 4-8h, providing evidence that the rate of production of hydrogen ions was maintained. It is apparent that *Homarus* achieves a better regulation of its acid-base state on exposure to air than does *Jasus*. Indeed, while $[H^+]$ more than doubled in *Jasus* after 8h, it had increased by only 40% in *Homarus* after 14h (Taylor and Whiteley, 1989).

The differences in acid-base regulation between *Jasus* and *Homarus* relate to the changes observed in PCO_2, lactate and $[HCO_3^-]$. Even disregarding the different air exposure times, in *Homarus* $[HCO_3^-]$ increased by 7.2 $mmol.l^{-1}$ compared to a 3.4 $mmol.l^{-1}$ rise in

Jasus, while the absolute changes in PCO_2 and [lactate] were nearly identical in the two species. Thus, *Homarus* has twice the bicarbonate buffering power of *Jasus* with which to counteract acid production.

Analysis of the pH-bicarbonate diagram indicates that during the first hour of emersion the drop in pH could be almost entirely explained by the increase in CO_2, since the change occurs directly on the buffer line. The concentration of lactate at that time was already 0.5 $mmol.l^{-1}$ higher than the resting concentration. This suggests that either hydrogen ions did not accompany the movement of lactate into the haemolymph or buffer base was rapidly added to the haemolymph on exposure to air. Indeed, comparison of the change in metabolic acid load with that of lactate (Fig. 4.9), shows there are considerable qualitative and quantitative discrepancies. Traditional concepts tend to view anaerobic metabolism as producing lactic acid which then dissociates into lactate anions and associated metabolic hydrogen ions. More recently it has been recognised that the metabolic H^+ are released during the hydrolysis of ATP. Under most circumstances the overall stoichiometry is for the release of H^+ and lactate ions in equimolar quantities (e.g. Zilva, 1978; Hochachka and Mommsen, 1983; Portner et al., 1984). Such a 1:1 relationship was not observed in the haemolymph of *Jasus*. Over the first 4h of emersion ΔLa^- increased while ΔH^+_m decreased, although at 8h ΔH^+_m once again approached zero (Fig. 4.9). The maximum disparity was observed at 8h emersion when the difference between the means was 4.6 $meq.l^{-1}$. It is therefore apparent that either hydrogen ions were removed from the haemolymph (or not released from the tissues) or alternatively, that the concentration of haemolymph buffers was raised.

A number of authors have noted that air-breathers lack access to an 'ion sink' in the form of external sea water. However, it was suggested that these animals mobilise an internal source of buffer, a possible site being the carapace (deFur et al., 1980; Henry et al.,

1981). Calcium carbonate released from the carapace and/or gut would be almost completely dissociated at this physiological pH, providing a potential source of base. Calcium did rise during emersion, although the change was relatively small. Comparison of the mean changes in haemolymph [Ca^{2+}] with the difference between the acid and lactate loads (i.e. $\Delta La^{-} - \Delta H^{+}_{m}$) indicates that only 25-30% of the 'additional' base could have been derived from a potential calcium carbonate release. This leads to the conclusion that either there is some additional source of base released into the bloodstream, possibly through intercompartmental ion exchange, or that Ca^{2+} is selectively removed from the haemolymph. In addition, hydrogen ions may be retained in the intracellular compartment. Aickin and Thomas (1975) determined the intracellular buffering capacity of crab muscle as 47 $meq.l^{-1}(pH\ unit)^{-1}$, or about 5 times higher than that determined here for *Jasus* haemolymph. Internal retention of hydrogen ions, therefore, may be less detrimental to the overall acid-base status of the animal. However, Mainwood and Worsley-Brown (1975) demonstrated preferential efflux of hydrogen ions from isolated muscle preparations in the frog when the pH of the bathing medium was low, exactly the reverse response to that observed here. Movements of bicarbonate between the intra- and extracellular compartments have been proposed by a number of authors who have recorded similar discrepancies between the acid and lactate loads.

From his analysis of the factors responsible for changing pH, Stewart (1978, 1981) concluded [H^{+}] could be altered by 2 major pathways: firstly, elevation of blood CO_2 will result in a respiratory acidosis; and secondly, by changes in the strong ion difference ([SID]). Because of the requirement for electroneutrality, changes in [SID] will be reflected in changes in the concentration of bicarbonate, according to the equation:

$$\Sigma[\text{strong cations}] - \Sigma[\text{strong anions}] = [HCO_3^{-}] + [Pr^{-}]$$

Assuming that [Pr^-] remains constant, an apparent rise in [HCO_3^-] is, according to Stewart's analysis, actually an increase in the net positive charge of strong ions. Ion exchanges have been well documented for both fish and crustaceans (see review by Kirschner, 1979). Although changes in the concentrations of strong ions (principally Na^+ and Cl^-) may occur either through co-transport of ions of the opposite charge (e.g. moving both Na^+ and Cl^-) or via countertransport of similarly charged ions (e.g. Cl^- exchanged for HCO_3^-), only the latter route can influence acid-base balance. Burnett and McMahon (1987) suggested that water retained within the branchial chambers could be used for ion exchange with the haemolymph in emersed animals. However, the number of moles of bicarbonate available for exchange from branchial water would be much lower than the bicarbonate increase observed in the haemolymph. Ion movements, therefore, must be restricted to the intra- and extracellular compartments. Thus the bicarbonate increase observed here which can not be accounted for by calcium carbonate release may well be the result of intercompartmental Cl^-/HCO_3^- exchange. Reduced [Cl^-] has been measured for *Austropotamobius* (Taylor et al., 1987) and *Homarus* (Taylor and Whiteley, 1989), and it has been proposed by these authors that ion exchange between the cells and haemolymph occur in these species during emersion.

While buffer base continued to rise between 0 and 4h emersion, by 8h the concentration once again approached that predicted by a respiratory acidosis along the buffer line. Bicarbonate concentration remained the same between 4-8h, suggesting that there was an effective limit to the ability with which *Jasus* could increase the buffering power of the haemolymph.

During reimmersion the changes that occurred in the haemolymph during emersion were rapidly reversed, with the exception of haemolymph lactate which took a full 24h before it was restored to

baseline levels. Oxygen consumption was considerably elevated over both the pre-emersion value and that recorded at the end of the emersion period, not returning to normal until 8-24h reimmersion. A considerable oxygen debt was incurred during the air-exposure period (Fig. 4.11). Assuming that the resting metabolic rate was maintained during emersion, the reduction in $\dot{M}O_2$ during exposure to air produced a measurable O_2 debt of about 1.85 mmol $O_2.kg^{-1}$. Comparison with the mean changes in haemolymph [lactate] revealed that the shortfall was made up by anaerobic metabolism. Estimates of the lactate contribution to the O_2 debt based on the change in haemolymph lactate, and assuming an even distribution throughout the body fluids as described in Chapter 3, indicated that 20% of the oxygen consumed during recovery (~7.9 mmol $O_2.kg^{-1}$) was used in lactate removal. Since lactate is apparently not excreted by crustaceans (Phillips et al., 1977) its disappearance must relate either to its oxidation to pyruvate or to its metabolism to CO_2. It may be that the lactate portion of the total debt was underestimated by assuming that the lactate concentration of the haemolymph was representative of the concentration in the whole animal. In the high shore crab *Leptograpsus variegatus* whole body lactate was measured at 12-100 times higher than haemolymph lactate (P. Greenaway, pers. comm.). Haemolymph [lactate] may not, therefore, provide a reliable estimate of total anaerobic metabolism, and hence the lactate part of the oxygen debt.

The increase in $\dot{M}O_2$ after emersion was enabled by a rapid increase in branchial water flow, as evidenced by the rise in f_{sc}, and presumably by an increase in gill perfusion, heart rate also rising to 50% higher than the pre-emersion rate. However, neither organ reached its maximal rate immediately on entry to water, maximum f_{sc} occurring at 1h reimmersion and heart rate slightly later at 2h. The reason for this delay is unknown. Perhaps there is a detrimental

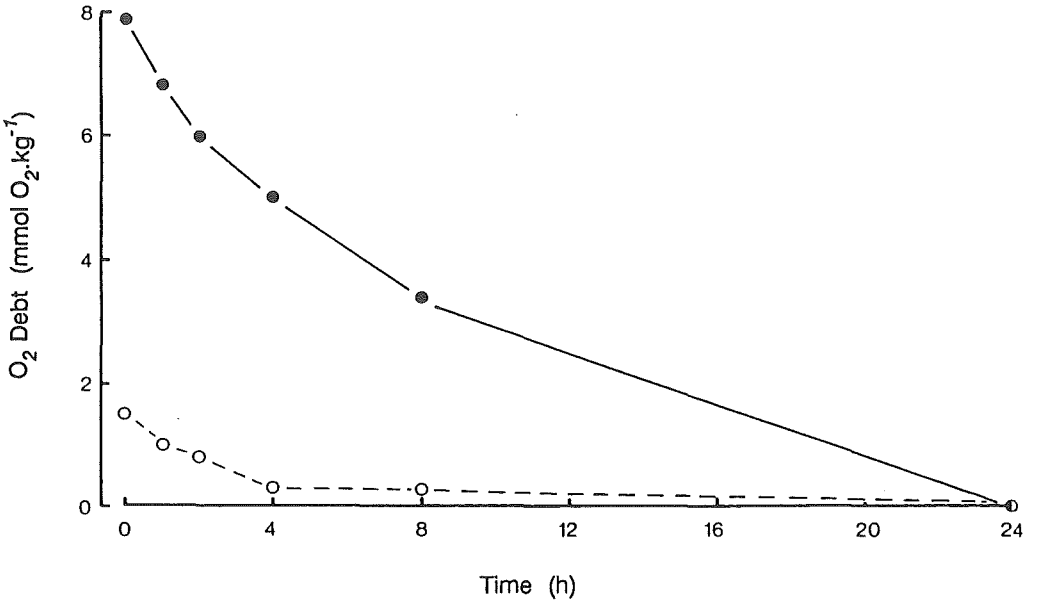

Fig. 4.11 Changes in the mean oxygen debt (●——●) and the estimated lactate component of the debt (○--○) during recovery from emersion in *Jasus edwardsii* at 17°C. The oxygen debt was calculated from the data given in Fig. 4.1, and the lactate component from the data in Fig. 4.7. Further details of the Δlactate estimate calculations are given in the text.

effect of air exposure on the receptor and pacemaker systems governing the action of both the scaphognathites and the heart. Vermeer (1987) found that emersion produced an impairment of the escape response (tail-flipping) in the spiny lobster, *Panulirus argus*, and concluded that this aberrant behaviour resulted from detrimental effects of hypoxia, an acidosis, and osmotic imbalance, on nervous tissue. It is possible the *Jasus* also sustained similar ill effects as those encountered by *Panulirus*, which may have resulted in the response lag in the respiratory and circulatory responses. Such nervous tissue damage would likely be the cause of the mortality noted earlier, since it was apparent that the animals which died were particularly listless and showed little response to external stimulation.

The gills revert back to their original physical state on return to water. CO_2 would thus diffuse down the elevated partial pressure gradient, resulting in its rapid removal from the haemolymph. Excretion of CO_2 would also be enhanced by the faster actions of the scaphognathites and heart, maximising the gradient driving CO_2 release. The reduction in PCO_2 obviously assisted in the restoration of haemolymph pH. About 25% of the excess hydrogen ions were lost within the first hour, and over this period PCO_2 dropped by 70% from the 8h, emersed value. However, restoration of haemolymph pH was delayed due to the loss of bicarbonate from the system. Part of the bicarbonate loss may have occurred through the action of gill carbonic anhydrase dehydrating bicarbonate to CO_2 which then diffused across the gills (McMahon et al., 1984; Burnett and McMahon, 1985). Alternatively, ion exchange of bicarbonate for seawater chloride ions may have occurred (Henry and Cameron, 1983; Taylor and Whiteley, 1989). In addition, although the changes in haemolymph $[Ca^{2+}]$ were generally not statistically significant, the concentration of calcium also decreased during the first part of recovery. Presumably in

conjunction with its removal, bicarbonate was withdrawn at the same time, a factor apparently ignored by other authors. However, between 1 and 2h into recovery approximately twice as much bicarbonate as calcium was lost, suggesting that this mechanism could not solely account for the decrease in $[HCO_3^-]$.

Haemolymph pH was essentially restored back to pre-emersion levels by 4h reimmersion, but even after that time it continued to rise slowly during further recovery in water. This slow phase of recovery was associated with a further reduction in haemolymph carbon dioxide tension as well as continued removal of lactate from the haemolymph.

In summary, *Jasus* responds to air exposure by a reduction in oxygen uptake, despite an increase in f_{sc}. This implies that efficient gas exchange could not occur, possibly through collapse of the gills when in air. The reduction in $\dot{M}O_2$ below the aquatic rate necessitated a shift towards anaerobic metabolism, increasing the haemolymph concentration of lactate. Emersion also induced an increase in haemolymph PCO_2, which, together with the metabolic acidosis, resulted in a progressive acidification of the haemolymph. Some compensation was achieved, although the change was small, and relatively short-lived.

Perhaps the most influential factor which disturbed haemolymph acid-base status during emersion was the 'loss' of the gills when in air. Since they are involved in both respiration and ion exchange under aquatic conditions, inhibition of their normal function will remove a major compensatory site for acid-base disturbances unless the animal is specifically adapted to cope with air exposure. That *Jasus* was unable to restore haemolymph acid-base status during emersion must reflect the stability of its usual habitat. In this respect, species such as high shore crabs (Truchot, 1975b; Innes et al., 1986; Burnett and McMahon, 1987) and *Austropotamobius* (Taylor

and Wheatly, 1980) that live in potentially unstable environments, would be expected to be better adapted to deal with changing acid-base conditions imposed by their environments.

CHAPTER 5

THE EFFECTS OF EXERCISE IN AIR, AND SUBSEQUENT EMERSION AND REIMMERSION, ON RESPIRATION AND HAEMOLYMPH ACID-BASE STATUS IN *JASUS EDWARDSII*

ABSTRACT

Changes in respiratory and circulatory variables and in haemolymph acid-base status in *Jasus edwardsii* following brief, strenuous exercise in air (50 tail-flips), during an initial post-exercise phase of 8h in air, and during a subsequent 48h period in water were measured at 17°C. Haemolymph pH fell most rapidly over the first hour and continued to decrease slowly for the whole of the aerial phase. Over the 8h pH fell from 7.52 in animals at rest in water, to 6.91 at the end of the emersion period. The acidosis was associated with progressive increases in PCO_2 and [lactate], which also changed most rapidly in the first hour. PCO_2 increased by 6.6 Torr and lactate by nearly 7 $mmol.l^{-1}$. The total scaphognathite rate (f_{sc}) rose from 150 bpm at rest to 260 bpm immediately after exercise, before falling to a level at 8h post-exercise only 45 bpm higher than in resting, submerged lobsters. Heart rate decreased initially, reaching a minimum at 1h, and remained below the submerged, pre-exercise rate for the whole post-exercise period in air. Similarly, oxygen uptake ($\dot{M}O_2$) was depressed to less than 40% of aquatic MO_2 throughout the aerial, post-exercise phase.

Haemolymph bicarbonate changed little in air. There was a small rise in haemolymph calcium and ammonia levels. Comparison of the metabolic acid load (ΔH^+_m) estimated from the pH-bicarbonate diagram with the change in lactate concentration showed that there was close

matching of these variables up to 4h in air, but at 8h ΔLa^- was 2.5 $meq.l^{-1}$ higher than ΔH^+_m. The dynamics of lactate and metabolic hydrogen ions are discussed.

Returning *Jasus* to water produced secondary increases in $\dot{M}O_2$, fsc and f_H, which peaked at 1h, 2h and 8h, respectively. This implies that oxygen uptake during recovery was enhanced by factors in addition to higher frequencies of the heart and scaphognathites. Nevertheless, their high rates were effective in reducing CO_2, since PCO_2 fell very rapidly and after 8h induced a very slight respiratory alkalosis. Haemolymph lactate levels remained significantly elevated until 24h. Since haemolymph pH was restored, this suggests that metabolically produced hydrogen ions were excreted or compensated independently of lactate. It is concluded that *Jasus* undergoes a considerable disruption to its normal acid-base and respiratory physiology when it is deprived of water after exercise. Apparently the mechanisms used for acid-base compensation following exercise in water are inoperative or do not operate appropriately in air.

INTRODUCTION

A number of studies have examined the influence of exercise on water- and air-breathing crustaceans. Species exercised in their normal respiratory medium generally possess mechanisms which lessen the impact of a severe period of activity. The gas exchange system increases oxygen transport into the animal and rapidly eliminates CO_2. Exercise also produces a significant change in the acid-base status of crustacean haemolymph. Most results indicate that the acidosis originates from increases in both PCO_2 and metabolic acids, the latter principally in the form of lactic acid (e.g. Booth et al., 1982, 1984; McDonald et al., 1979; Smatresk et al., 1979; Wood and

Randall, 1981a). Recovery may take several hours and is effected by ventilatory adjustments, remetabolism of lactate and ion exchange mechanisms. Despite a wealth of information of the effects of exercise of aquatic crustaceans in water, there have been no previous studies which have examined the effect of exercise in air and the extent of recovery possible in a subtidal animal during a prolonged post-exercise period in air.

It was shown in the two previous chapters that *Jasus* employs different mechanisms for regulating the acid-base status of its haemolymph following exercise in water and during emersion. Part of this difference related to the influence of the two media on oxygen uptake. In water the oxygen debt imposed by a heavy exercise regime was able to be repaid relatively quickly. In contrast, *Jasus* was unable to sustain even its standard level of oxygen uptake during emersion, leading to a progressive, combined respiratory and metabolic acidosis. The oxygen debt and acidosis experienced by *Jasus* on emersion were somewhat more severe than the changes reported for another sub-tidal, marine macruran, *Homarus gammarus* (Taylor and Whiteley, 1989).

The problems associated with activity, such as adequate delivery of oxygen to the tissues and removal of metabolically produced carbon dioxide, are in themselves sufficient to result in a disruption of the animal's routine level of metabolism. They may also result in considerable displacement in haemolymph pH. When combined with a lack of the usual respiratory medium, the difficulties associated with exercise are liable to become even more pronounced.

It was inferred from the evidence presented in the two previous chapters that following either exercise or emersion there was a shortfall in oxygen supply to the tissues, resulting in the development of an internal hypoxia. There was a rapid increase in scaphognathite frequency in both experiments, although heart rate

increased after exercise, but decreased initially on emersion.

Whereas the previous chapter dealing with exercise examined the physiological consequences of exercise in water, the experiments discussed here relate to the changes occurring in *Jasus* after it was exercised and held in air, followed by a recovery period in water. The aim of these experiments was to characterise the changes arising from this protocol, and more specifically to compare the changes with those recorded during exercise in water and those resulting from emersion alone.

MATERIALS AND METHODS

Rock lobsters of either sex and weighing between 270 and 650 gms were mainly collected off the Canterbury coast, although some animals were obtained from a commercial supplier in Southland. The animals were transported to the laboratory where they were held in recirculating sea water (salinity ~ 36°/oo) at 17 ± 2°C for several weeks before experimentation.

Experimental procedure

To facilitate comparison, the protocol used was similar that of the emersion experiments (Chapter 4), except that at the beginning of emersion the animals were exercised. As in the previously described experiments, the rock lobsters were set up in respirometers or were implanted with electrodes at least 48h before experimentation. Control values were measured at the end of the settlement period. The experimental chambers were drained and the animals exposed to air. Exercise was similar to that in Chapter 3, although the animals were first removed into air. Within approximately 30 seconds of the chambers being drained the exercise procedure was initiated. Tactile

stimulation and handling were usually required to elicit 50 tail-flips. The exercise period was again short and took between 1-4 minutes to complete.

Following exercise the rock lobsters were held in air for 8h (designated Stage I post-exercise, or post-exercise emersion) before water was restored and the changes measured over the following 48h (Stage II, or reimmersion post-exercise).

Experimental protocol and analytical techniques

The three experimental series used in the exercise and recovery in water experiments (Chapter 3) and the emersion experiments (Chapter 4) were again used here. In Series I oxygen uptake was measured on 9 animals using the closed box method described previously. The animals were transferred to watertight, plastic boxes and $\dot{M}O_2$ calculated on the basis of the respirometer volume and the change in P_IO_2 over a known time interval after correcting for changes in PO_2 resulting from O_2 uptake by micro-organisms. During Stage I (air exposure) the PO_2 changes were smaller so the time during which the respirometers were sealed was longer. Measurements were made at 2-hourly intervals after 2, 4, 6 and 8h emersion. The results have been graphically presented over each of these time intervals, and are referred to in the text as 1, 3, 5 and 7h post-exercise. To minimise visual disturbances the respirometers were covered with black plastic sheeting.

Series II consisted of measurements of heart (f_H) and total scaphognathite rates (f_{sc} = sum of the left and right scaphognathites) made on 8 animals using the impedance technique described in Chapter 2. Briefly, this consisted of implanting paired, silver wires in holes drilled through the carapace on either side of the heart or scaphognathites. The change in impedance caused by their beating was detected by impedance coupler units (Strathkelvin,

Biosciences A100) and the signal recorded on a Gould recorder.

Changes in acid-base state resulting from exercise in air and then recovery were made on a total of 26 rock lobsters (Series III). During each experimental run the animal was sampled at only one time interval before being returned to water for at least 48h before another experimental test was begun. Thus, although several samples were made on each individual animal, repetitive sampling throughout the course of an experimental run was avoided. At each time interval during this set of experiments the data are given as the means from 8 animals. Pre-branchial haemolymph was sampled anaerobically (as described in previous chapters) with a fine (20 gauge) needle via the arthrodial membrane at the base of the legs. A small sample was first removed to fill the dead space in the syringe. Total sampling time was less than 40 seconds.

Each blood sample was divided into aliquots for measurements of pH, total CO_2 (CCO_2), lactate, ammonia, calcium and osmotic pressure. The methods used have been described in more detail in previous chapters. Subsamples were removed immediately for the determination of pH and CCO_2. Haemolymph pH was measured on 70 μl samples using a Radiometer G297/G2 microelectrode at 17 ± 1°C, standardised with precision buffers (Radiometer S1500 and S1510). Total CO_2 was determined using the method of Cameron (1971) on 20 μl samples with a PCO_2 electrode (Radiometer 5036) connected to a PHM 84 meter. Calibration was performed using 20 μl samples of 10 mmol.l^{-1} $NaHCO_3$.

Haemolymph lactate concentration was determined enzymatically with a Boehringer food analysis kit for lactate which had been modified to prevent the interference of Cu^{2+} ions as detailed by Engel and Jones (1978). One hundred and fifty microlitre haemolymph samples were deproteinated in 300 μl of ice cold, 0.6N $HClO_4$ and then frozen before analysis.

Haemolymph ammonia levels were determined by adaptation of a

Boehringer test-combination kit for urea. Twenty microlitre samples were added to the base solutions and the change in absorbance determined spectrophotometrically (Kontron Uvikon 860).

Changes in haemolymph calcium were measured using atomic absorption spectrophotometry (Varian Techtron 1200) and osmotic pressure using a Wescor 5100C vapour pressure osmometer.

The value determined *in vitro* in Chapter 3 for the slope of the non-bicarbonate buffer line was used in the construction of the pH-bicarbonate diagram.

Changes in PCO_2, $[HCO_3^- + CO_3^{2-}]$, ΔH^+_m, and ΔLa^- were calculated using the equations given in Chapter 3.

Statistical treatment

All data are given as the means ± 1 S.E.M. A GLM ANOVA was initially used to compare the data sets and then Fisher's Least Significanct Difference Test to determine significant differences between the means. Significance was designated at the 95% confidence interval ($p<0.05$).

RESULTS

Respiratory Variables

The effects of exercising *Jasus* in air, followed by 8h in air (Stage I) and then a further 48h in water (Stage II), on oxygen consumption, ventilation frequency (f_{sc}) and heart rate (f_H) are shown in Figs. 5.1-5.3.

In settled submerged animals $\dot{M}O_2$ was 13.53 ± 1.12 $\mu mol.kg^{-1}.min^{-1}$ which was not significantly different to any of the values measured previously ($p>0.05$). Despite an expected increase in the requirement

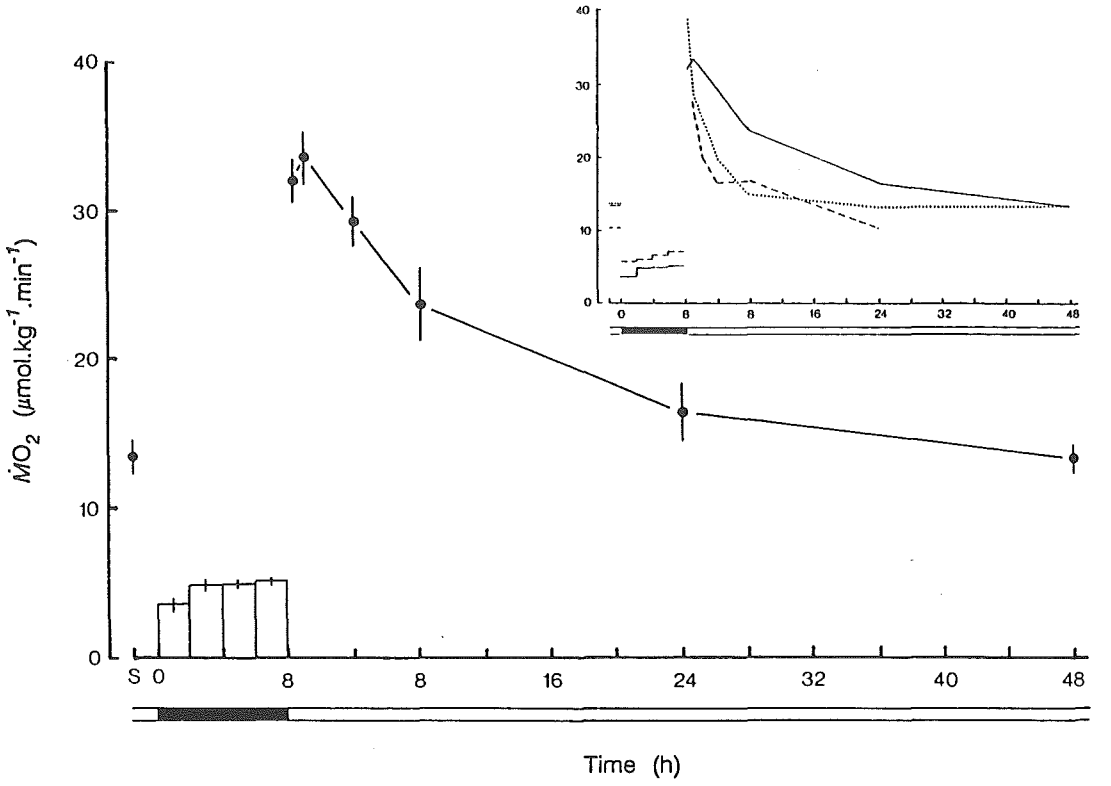

Fig. 5.1 The effect of exercise in air, emersion after exercise, and subsequent reimmersion, on oxygen uptake ($\dot{M}O_2$) in *Jasus edwardsii* at 17°C. Initial resting values (S) were determined after 48h in water. The horizontal bar beneath the time base indicates time in water (unshaded) and time in air (shaded). The histogram arrangement of the data during the emersed post-exercise phase represents mean oxygen uptake determined over 2h intervals. Data are given as the mean ± 1 SEM, n = 9. ***Inset*** shows the pattern of changes occurring in $\dot{M}O_2$ after exercise in water and undisturbed emersion for comparison. Note that $\dot{M}O_2$ following exercise in water has been displaced to coincide with the reimmersion data obtained from these experiments and those of Chapter 4. Solid line = exercise in air; broken line = undisturbed emersion; dotted line = exercise in water.

for oxygen following work above the anaerobic threshold, $\dot{M}O_2$ decreased significantly from the control value during the first post-exercise (air) period (3.63 ± 0.45 μmol.kg^{-1}.min^{-1} at 1h post-exercise in air, $p<0.001$, Fig. 5.1). Although $\dot{M}O_2$ increased somewhat in air, even at 6-8h post-exercise it was still highly depressed (mean value = 5.15 ± 0.24 μmol.kg^{-1}.min^{-1}, $p<0.001$) compared to the resting oxygen consumption.

When *Jasus* was returned to sea water to recover from the combined effects of exercise and emersion, there was a large and rapid increase in oxygen uptake. The peak value at 1h was 33.6 ± 1.8 μmol.kg^{-1}.min^{-1}, a 2.6-fold increase over resting $\dot{M}O_2$ and a 6.5-fold increase over the final value at 6-8h post-exercise in air. With further time in water oxygen consumption gradually decreased so that by 24h into this recovery phase $\dot{M}O_2$ was not significantly different from resting $\dot{M}O_2$.

Although $\dot{M}O_2$ decreased during the post-exercise, emersed phase, the total scaphognathite rate (f_{sc}) increased immediately after exercise (Fig. 5.2), rising significantly from approximately 150 bpm at rest to 260 bpm within the first 15 minutes ($p<0.02$). Subsequently f_{sc} decreased and by 4-8h post-exercise in air it was only about 45 bpm higher than the resting value. The large variability in the samples meant neither the 4h nor 8h values was significantly different from the resting, aquatic ventilation frequency. When *Jasus* was returned to water (Stage II) f_{sc} rose in a similar fashion to $\dot{M}O_2$, although the peak rate, measured at 2h, occurred slightly later than $\dot{M}O_2$, when f_{sc} was 352 ± 22 bpm ($p<0.002$). Reduction back to the pre-exercise level also took longer than the restoration of $\dot{M}O_2$, since f_{sc} at 24h was still significantly elevated over the pre-exercise, immersed rate ($p<0.05$).

The pattern of changes in heart rate (Fig. 5.3) paralleled $\dot{M}O_2$ rather than f_{sc}, at least during the aerial, post-exercise phase.

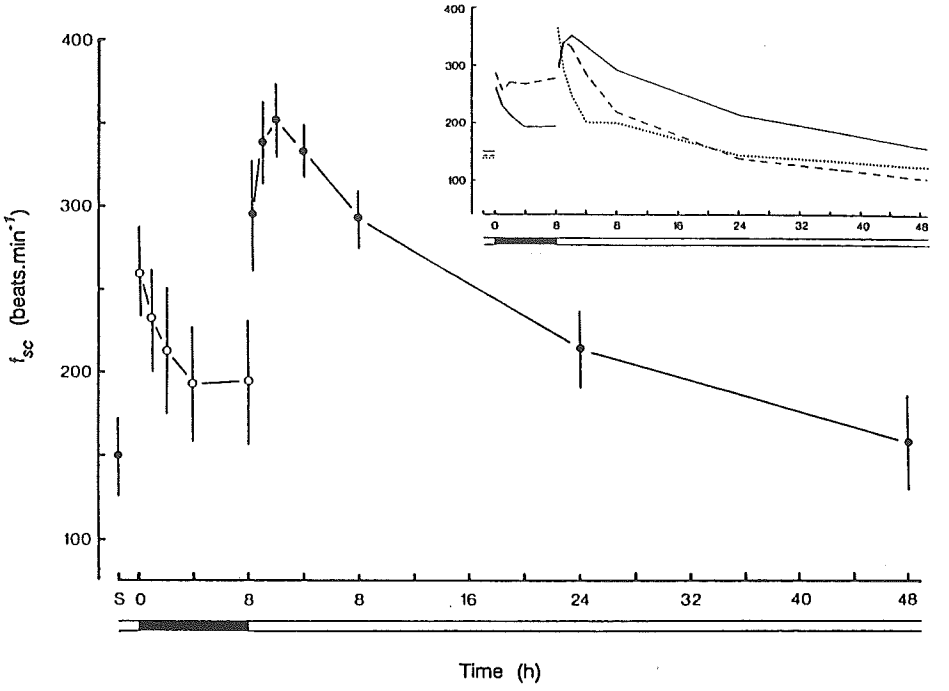

Fig. 5.2 Changes in respiratory frequency (f_{sc} = sum of the left and right scaphognathites) during emersion after exercise and subsequent reimmersion at 17°C. Open symbols refer to the post-exercise period in air and closed symbols represent measurements made in water. The emersed period is indicated by the shaded area of the bar below the time scale. Data are given as mean ± 1 SEM, n = 8. ***Inset*** shows the changes in f_{sc} following exercise in water (Chapter 3) and during emersion (Chapter 4) for comparison with the data obtained in these experiments.

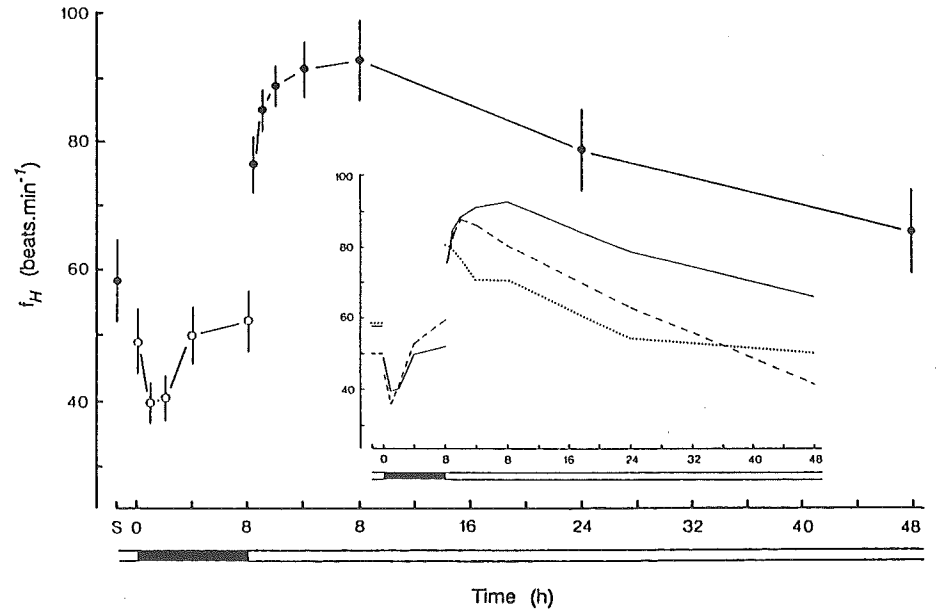

Fig. 5.3 The effect of exercise in air, post-exercise emersion and 48h reimmersion on heart rate (f_H) in *Jasus edwardsii* at 17°C. n = 8. Other details as given in Fig. 5.2.

Heart rate decreased immediately after exercise and by 1h had fallen by 18 bpm, a significant depression over the resting rate of 58 bpm ($p<0.02$). Later in recovery f_H rose, so that by 8h post-exercise the mean value was only 6 bpm slower than f_H pre-exercise. However, return to water produced a rapid rise of more than 24 bpm within the first 15 minutes. The peak rate occurred later than those of either $\dot{M}O_2$ or f_{sc} (92.6 ± 6.4 bpm after 8h in water). By 48h in water f_H was reduced to only about 8 bpm higher than normal, and there was no statistical difference between the heart rate at that time and f_H at rest.

Acid-Base Variables

Changes in pH, calculated PCO_2, bicarbonate ($[HCO_3^- + CO_3^{2-}]$), lactate and ammonia concentrations before and after exercise in air, and during the two post-exercise phases of 8h in air followed by 48h in water, are shown in Figs. 5.4-5.8.

Haemolymph pH immediately fell after exercise (Fig. 5.4). Within 15 minutes it had dropped by slightly more than 0.2 units over the resting value of 7.520 ± 0.021. Emersion after exercise continued to depress haemolymph pH, so that at 8h it was 6.907 ± 0.041 units, which represented a highly significant 4-fold increase in the concentration of hydrogen ions ($p<0.001$).

Returning *Jasus* to water rapidly reduced the extent of the acidosis. By 15 minutes reimmersion haemolymph pH had risen approximately 0.2 units from the 8h, emersed value (equivalent to a 40% reduction from the $[H^+]$ present at 8h in air). Despite the initially rapid rise in pH, it still took up to 8h to restore the mean haemolymph pH to about the same as - or even slightly (but non-significantly) higher than - the mean pre-exercise level.

Pre-branchial PCO_2 levels were generally quite low at rest (2.17 ± 0.20 Torr), but rose sharply after exercise, increasing by

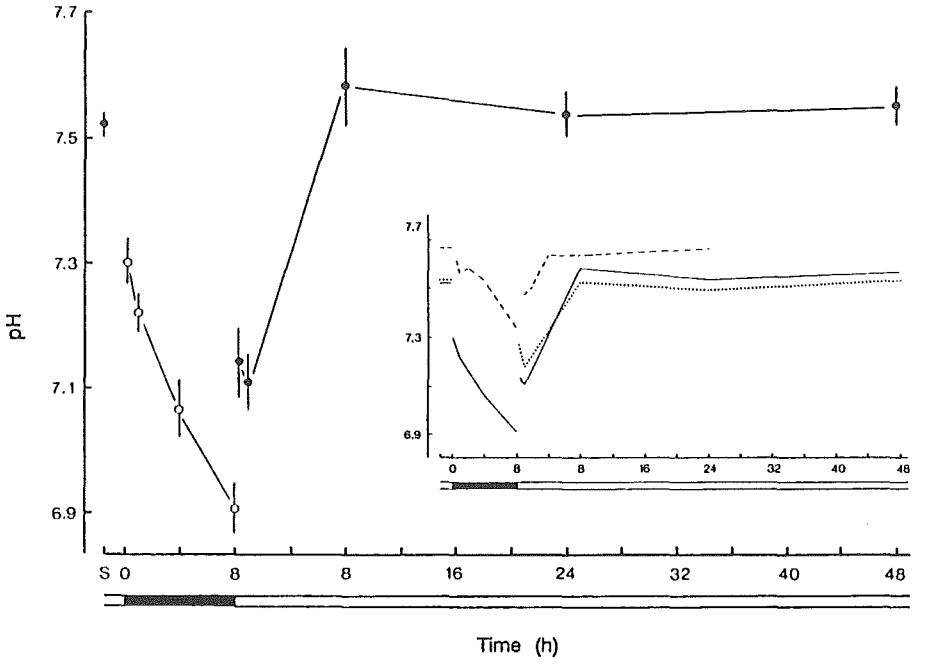

Fig. 5.4 Changes in pre-branchial haemolymph pH during emersion after exercise (open symbols) and subsequent reimmersion (closed symbols) at 17°C. n = 8. Other details as described in Fig. 5.2.

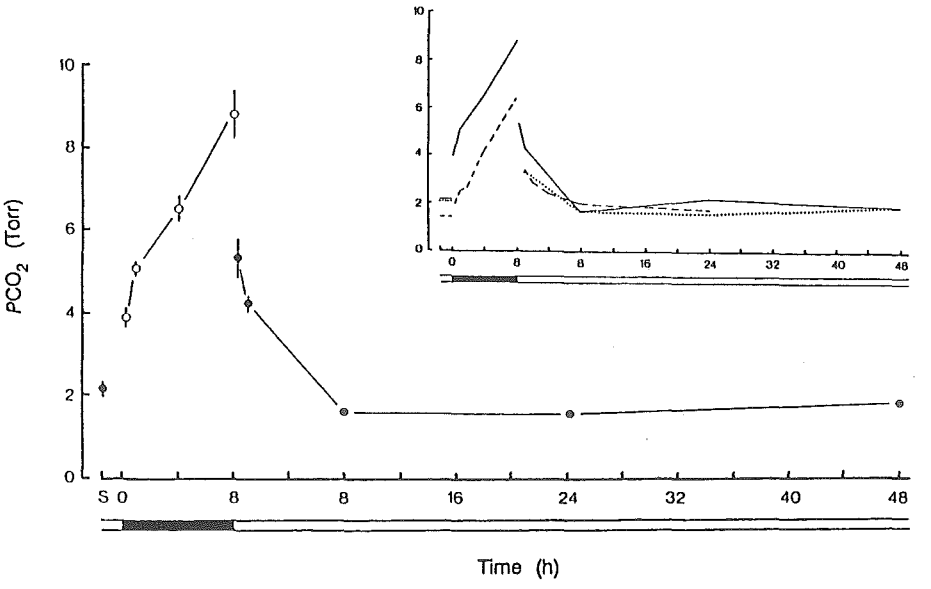

Fig. 5.5 The effect of exercise in air, post-exercise emersion, and reimmersion on calculated PCO_2 in *Jasus* at 17°C. n = 8. Other details as given in Fig. 5.2.

about 80% within the first 15 minutes post-exercise ($p<0.001$, Fig. 5.5). The longer *Jasus* remained in air the more PCO_2 increased. Although the rate of increase slowed somewhat later in the emersed phase, by 8h post-exercise PCO_2 had risen 4-fold to 8.82 ± 0.61 Torr ($p<0.001$, Fig. 5.5).

PCO_2 was rapidly reduced on reimmersion. Fifteen minutes in water saw a 3.5 Torr drop from the post-exercise peak recorded at 8h in air. At 8h reimmersion it had decreased to 1.67 ± 0.07 Torr, a value which was significantly lower than the pre-exercise tension ($p<0.02$). After 24h mean PCO_2 had decreased slightly further, and it was not until 48h that PCO_2 was restored to the pre-exercise, aquatic level.

Changes in the concentration of bicarbonate ($[HCO_3^- + CO_3^{2-}]$) during the aerial, post-exercise phase were quite small, with an increase of less than 1 $meq.l^{-1}$ over the entire 8h period (Fig. 5.6). None of the changes during the emersed phase was statistically significant.

Reimmersion produced a transient rise in bicarbonate at 15 minutes, which was succeeded by a small, and statistically significant, decrease at 1h when the bicarbonate concentration was 2.28 ± 0.22 $meq.l^{-1}$ ($p<0.05$). After that low it started to rise again and, apart from a small, but not significant, decrease at 24h, was back to about the resting concentration by 8 - 48h.

Haemolymph lactate concentration was low at rest (0.192 ± 0.027 $mmol.l^{-1}$, Fig. 5.7), but within 15 minutes post-exercise in air it had increased significantly by 0.8 $mmol.l^{-1}$ ($p<0.001$). Prolonged emersion after exercise served only to increase the amount of lactate in the haemolymph, so that by the end of the 8h post-exercise period in air mean [lactate] was 6.97 ± 1.36 $mmol.l^{-1}$, one animal reaching 15.3 $mmol.l^{-1}$. The concentration decreased as soon as the rock lobsters were reimmersed, [lactate] falling by approximately 1.7

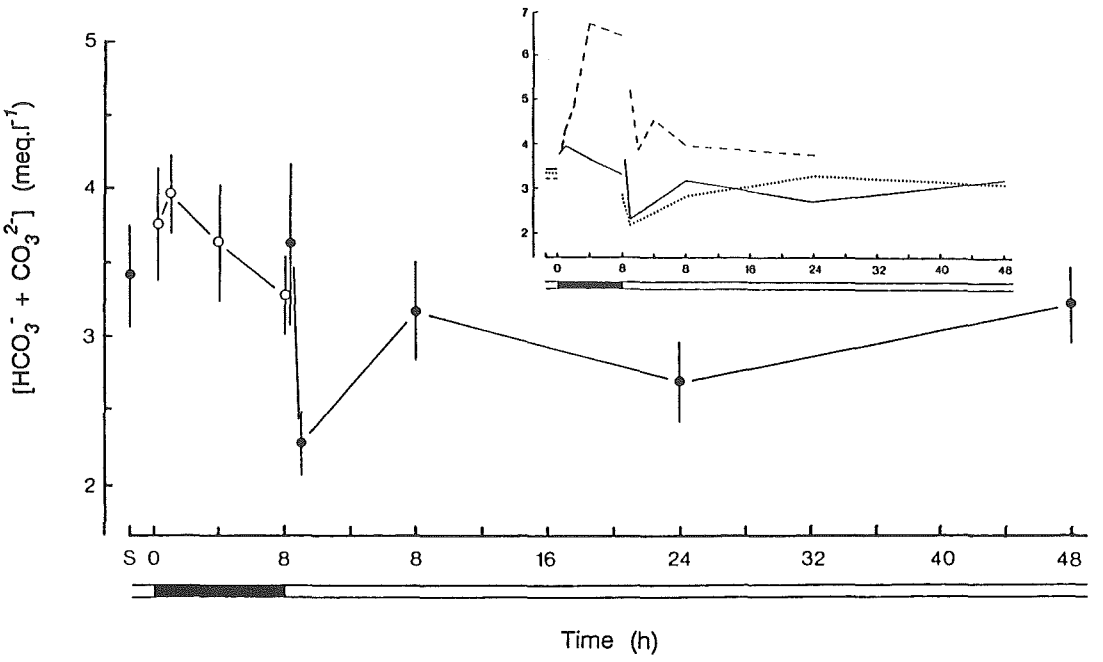

Fig. 5.6 Changes in bicarbonate concentration ($[HCO_3^- + CO_3^{2-}]$) in pre-branchial haemolymph during 8h emersion after exercise and during 48h subsequent reimmersion. n = 8. Other details as in Fig. 5.2.

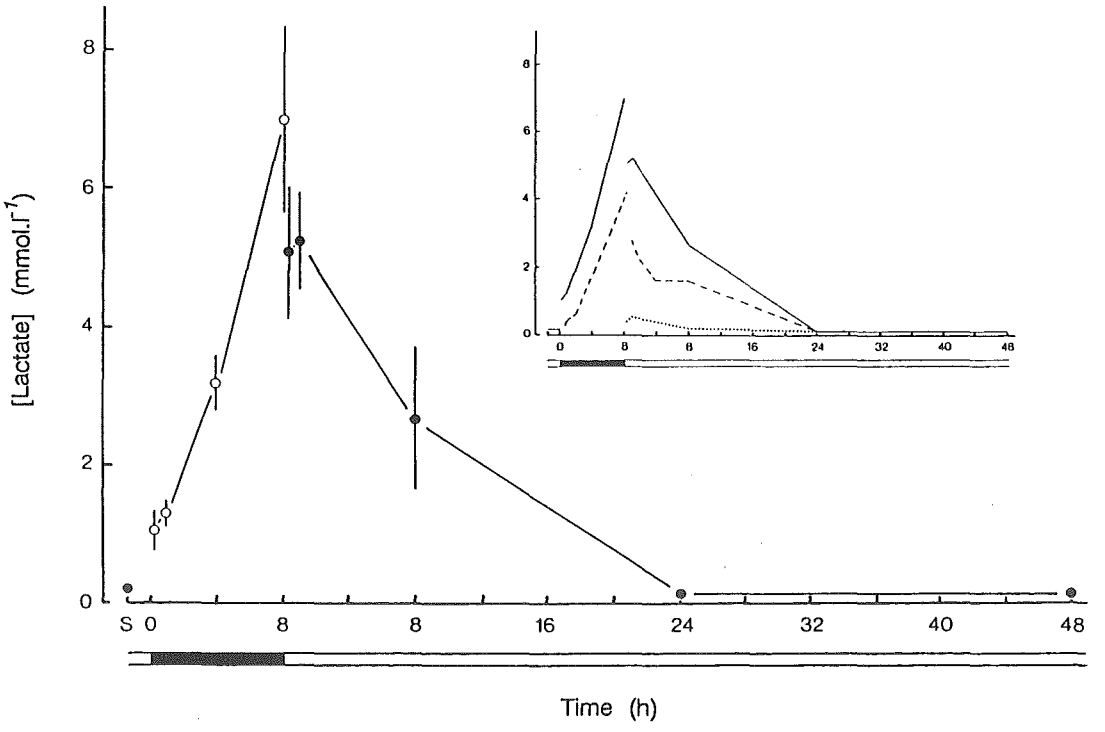

Fig. 5.7 The effect of exercise in air, emersion after exercise, and reimmersion on haemolymph [lactate] in *Jasus* at 17°C. n = 8. Other details are given in Fig. 5.2.

$mmol.l^{-1}$ within the first hour. While the level slowly continued to fall, it was still significantly elevated over the resting concentration after 8h in water ($p<0.02$) and it was not until 24h that it reached a level not significantly different from the pre-exercise concentration.

Changes occurring in haemolymph ammonia after exercise in air and during the two recovery phases are shown in Fig. 5.8. Although somewhat erratic, a very clear increase was recorded during the emersed, post-exercise period. At 15 minutes NH_3 had risen significantly by 0.3 $mmol.l^{-1}$ from the resting concentration of 0.44 ± 0.07 $mmol.l^{-1}$ ($p<0.002$). It continued to rise throughout the first post-exercise period, so that by 8h the concentration had risen to 1.161 ± 0.135 $mmol.l^{-1}$ ($p<0.001$).

Reimmersion had a similar effect on haemolymph ammonia as it had on the other variables described previously. The concentration decreased rapidly and fell by 0.3 $mmol.l^{-1}$ after 15 minutes in water. It continued to decrease during reimmersion so that it was not significantly different from the resting concentration by 1h in water.

Haemolymph Calcium and Osmotic Pressure

The total change measured in haemolymph calcium was small, the maximum mean change being less than 2.5 $mmol.l^{-1}$ (Fig. 5.9). Although none of the changes were statistically significant during the emersed phase, there was a general increase in haemolymph $[Ca^{2+}]$ when *Jasus* was held in air post-exercise. $[Ca^{2+}]$ increased from 15.33 ± 0.41 $mmol.l^{-1}$ pre-exercise to 16.71 ± 0.60 $mmol.l^{-1}$ after 8h in air. Returning the animals to water resulted in a further 0.5 $mmol.l^{-1}$ rise at 15 minutes (significantly different from controls, $p<0.02$) before the concentration began to decrease towards the pre-exercise concentration.

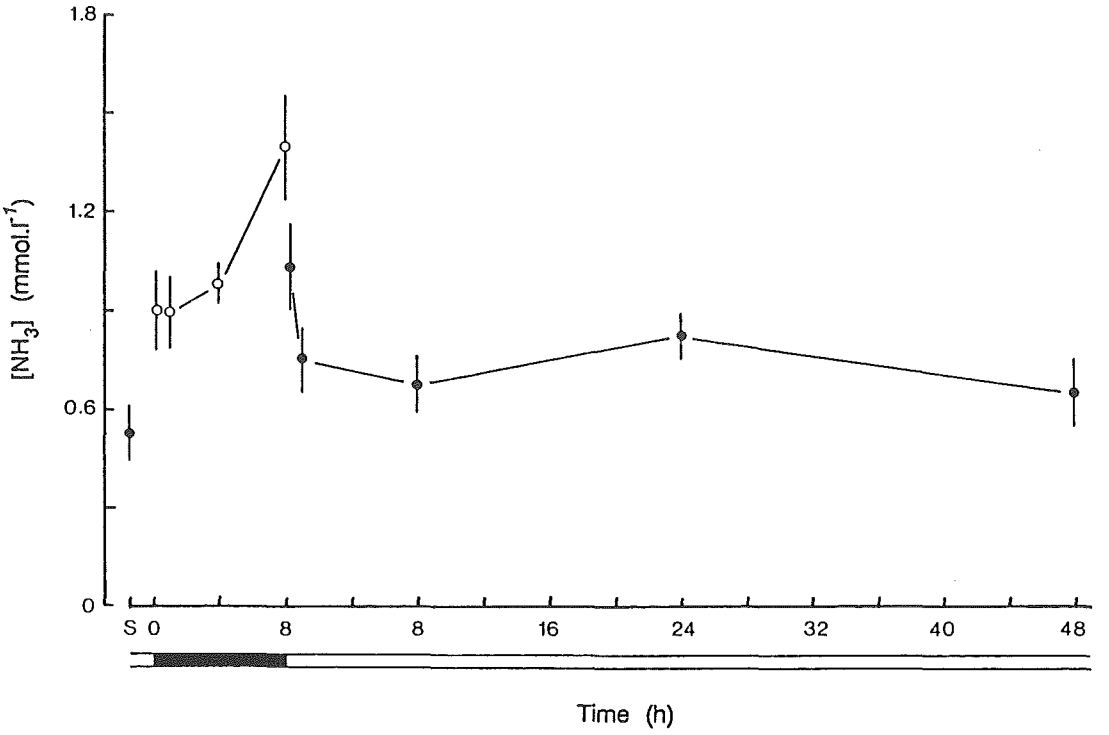

Fig. 5.8 Changes in $[NH_3]$ (= NH_3 + NH_4^+) in the haemolymph of *Jasus* during air exposure post-exercise, and subsequent reimmersion at 17°C. n = 8. Other details as in Fig. 5.2.

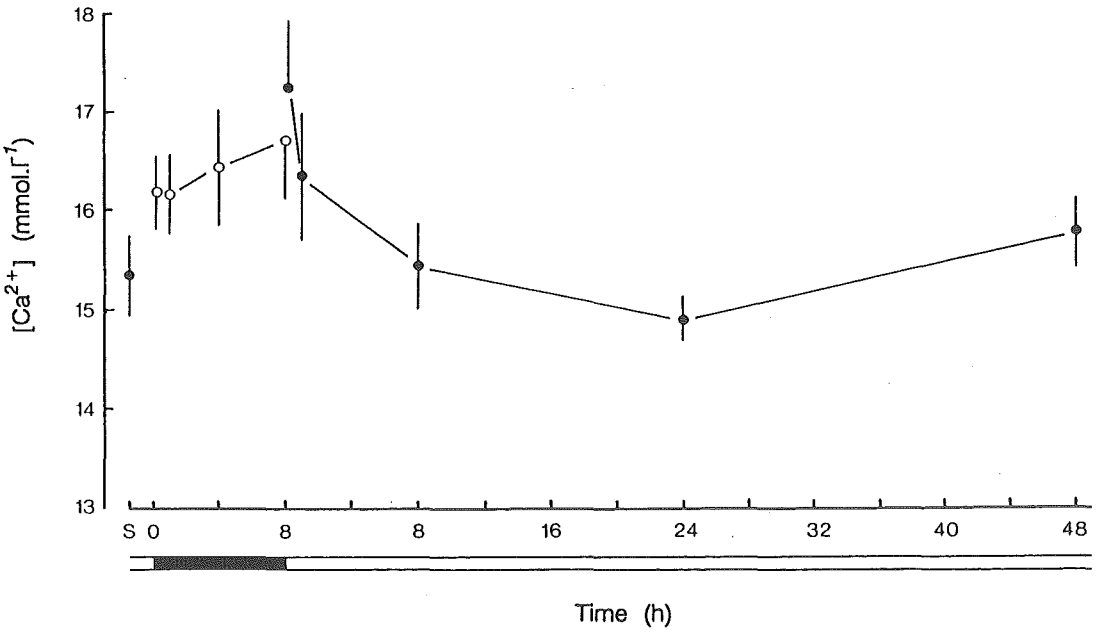

Fig. 5.9 Changes in the mean concentration of calcium in pre-branchial haemolymph during 8h emersion after exercise and 48h reimmersion. n = 8. Other details as in Fig. 5.2.

Haemolymph osmotic pressure rose from 1025 ± 7 $mOsm.kg^{-1}$ pre-exercise to 1037 $mOsm.kg^{-1}$ at 8h post-exercise, a change of about 1% which was not statistically significant.

Acid-Base Analysis

To estimate the relative influences of metabolic and respiratory acids in determining pH during aerial exercise and subsequent recovery 3 variables - pH, PCO_2 and $[HCO_3^- + CO_3^{2-}]$ - have been plotted on a pH-bicarbonate diagram (Fig. 5.10). The mean *in vitro* buffer line has a slope of -8.03 slykes and is the same as that determined in Chapter 3.

The graphical analysis (as detailed by Wood et al., 1977) reveals emersion following exercise produced a mixed respiratory and metabolic acidosis. Up to 1h post-exercise, respiratory (CO_2) acids were more important in determining the final pH value, contributing between 60 and 65% of the acid load at 1h. Between 4h emersion and 15 minutes reimmersion respiratory and metabolic acids contributed approximately equally to the acidosis. Reimmersion, however, initially resulted in a rapid increase in pH towards the pre-exercise value, indicating a decline in both acid sources. By 1h reimmersion there was a slight further reduction in the respiratory acid load but metabolic acids had increased, effectively stabilizing haemolymph pH to the state measured after 15 minutes in water. Later into recovery both sources were restored to about the pre-exercise levels.

DISCUSSION

It was shown previously (Chapter 4) that *Jasus* was unable to sustain aerobic metabolism in air, even at rest. However, some limited ability to reduce the overall magnitude of acid-base

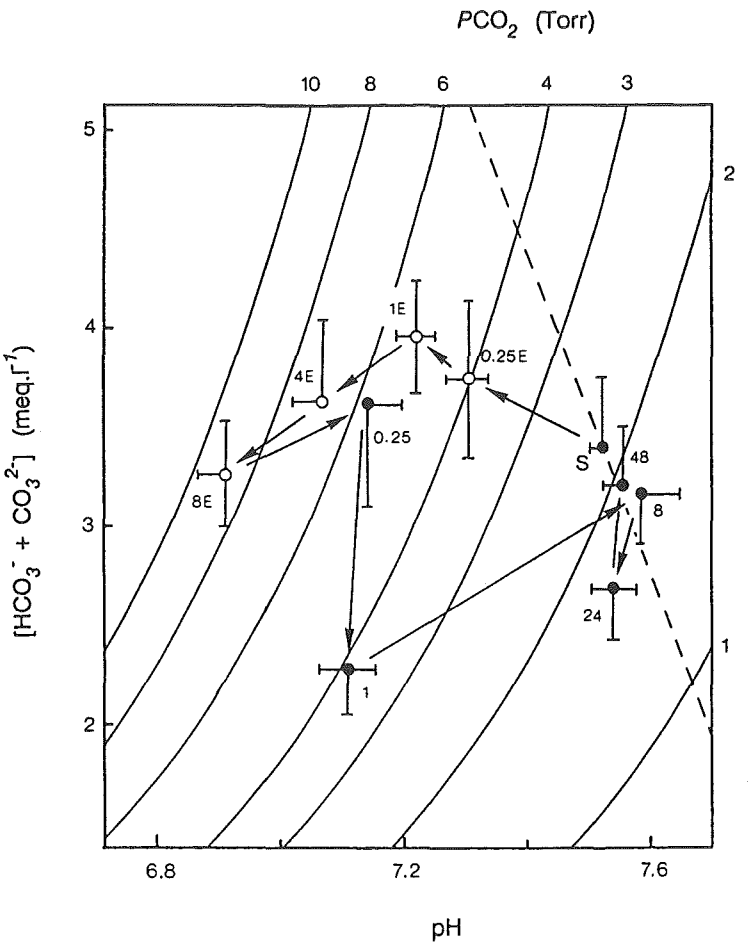

Fig. 5.10 Simultaneous changes in pH, $[HCO_3^- + CO_3^{2-}]$ and PCO_2 during post-exercise emersion (0.25E, 1E, 4E, 8E ○) and subsequent reimmersion (0.25, 1, 8, 24, 48 ●) in *Jasus edwardsii* at 17°C. S = initial, settled value. The dashed line represents the non-bicarbonate buffering capacity.

disturbances was noted. Despite rising haemolymph [lactate], the drop in pH was similar to that expected from respiratory sources alone. Indeed, up to four hours, part of the respiratory acidosis was compensated by a rise in [HCO_3^-], presumably from endogenous sources.

Combining exercise with emersion would be expected to exacerbate the respiratory and acid-base problems. This appears to be the case. The responses of *Jasus* under these conditions suggest that it is incapable of marshalling any further capacity for gas exchange or acid-base compensation above that observed in response to emersion alone. In fact the values recorded for $\dot{M}O_2$ post-exercise in air were actually lower than those seen in emersion alone. $\dot{M}O_2$ at 1h post-exercise was only 27% of resting, aquatic $\dot{M}O_2$ compared to about 56% in lobsters which were emersed only, and at 8h it had risen only slightly to about 40% of the resting $\dot{M}O_2$ (see inset, Fig. 5.1). Statistical comparison of the changes in $\dot{M}O_2$ between the exercised/emersed and non-exercised/emersed lobsters, using individually normalised values with each animal acting as its own control, indicated that a highly significant difference existed between the 2 groups ($0.01<p<0.004$). This response to emersion is clearly counter to the metabolic requirements of the animal immediately post-exercise.

It Chapter 3 it was shown that exercise of fifty tail-flaps imposed a heavy metabolic demand on *Jasus*. Essentially the same regime was used to promote activity under aerial conditions. Although the resistance to tail flexion in air would be rather less than that in water, it is unlikely that the energetic cost of 50 tail flips would be substantially lower in air than in water. Whereas exercise in water resulted in a 25 $\mu mol.kg^{-1}.min^{-1}$ rise in $\dot{M}O_2$ and induced a small lactacidosis, after a similarly strenuous period of activity in air oxygen uptake was, in fact, reduced by 10 $\mu mol.kg^{-1}.min^{-1}$ (inset, Fig. 5.1). Comparing the changes in $\dot{M}O_2$ following exercise in the two

media indicates that the anaerobic component of exercise is likely to be much greater in air than water.

The greater anaerobic component of aerial exercise is supported by haemolymph lactate measurements. There was a 1 $mmol.l^{-1}$ rise in haemolymph lactate immediately post-exercise in air, compared to an increase of only 0.2 $mmol.l^{-1}$ in water (Chapter 3).

Part of the change in lactate observed over the 8h period must reflect the response to emersion itself. [Lactate] rose continuously throughout undisturbed air exposure, increasing by ~4 $mmol.l^{-1}$ compared to a total rise of 7 $mmol.l^{-1}$ after exercise (inset, Fig. 5.7). Thus, even disregarding the most rapid phase of lactate appearance between 0-15 minutes post-exercise, the rise in lactate following exercise exceeded that during emersion by 50%. During emersion lactate increased at a mean rate of 0.51 $mmol.l^{-1}.h^{-1}$, whereas it rose by 0.77 $mmol.l^{-1}.h^{-1}$ during the post-exercise period in air. Haemolymph [lactate] of rock lobsters held in air post-exercise was therefore higher than the summed effects of emersion and exercise in water.

The higher rate of appearance of lactate in the haemolymph during emersion following exercise, compared with that during un disturbed emersion may relate to one of several factors: (1) a general depression of the metabolic rate during undisturbed emersion (although this is perhaps unlikely since all animals used had been settled for 48h before treatment); (2) a delayed release of lactate from the exercised tail muscles into the haemolymph; or (3) a generally elevated metabolic rate associated with disturbance (or arousal) associated with the stimulus to exercise.

The higher rate of lactate appearance in the exercised rock lobsters may also relate to the changes observed in $\dot{M}O_2$. Emersion alone was accompanied by a marked depression in oxygen uptake and was followed by a partial recovery. A similar pattern was observed in the

exercised and air-exposed rock lobsters. However, in spite of the increased metabolic demands imposed by the exercise bout, $\dot{M}O_2$ over the post-exercise period in air was actually lower than that of the emersed lobsters.

When averaged over the whole eight hours, post-exercise $\dot{M}O_2$ was only 37% of aquatic, pre-exercise $\dot{M}O_2$, whereas that of the emersed-only group was nearly double this at 69%. This difference may result from ventilatory changes and from changes in gill perfusion. Ventilation frequency increased immediately on emersion in both the exercised and non-exercised rock lobsters. However, the increase was larger (~145 bpm, Chapter 4, also inset in Fig. 5.2) and sustained for longer in the lobsters emersed only than in those exercised and emersed (a rise of 110 bpm initially, Fig. 5.2). In Chapter 2 it was shown that ventilation volume increased predictably with an increase in ventilation frequency. Assuming that a qualitatively similar relationship exists in air, a lower f_{sc} implies a smaller volume of air passing through the branchial chambers of the exercised group. However, given the O_2 capacitance of air, this effect is probably of only minor importance in explaining the differences in $\dot{M}O_2$. In addition, heart rate also decreased further after exercise than it did during emersion, although the difference was, again, minor. Differences in f_{sc} and f_H appear proportionally too small to account for the larger drop in $\dot{M}O_2$ after exercise. Thus some other, at present unknown, factor must be involved in the greater reduction in oxygen uptake after activity.

A surprising effect during the post-exercise period in air was the pattern occurring in the rate of beating of the scaphognathites (Fig. 5.2) Scaphognathite rate increased initially after exercise before decreasing during the latter stages of air exposure, but remained elevated on emersion alone (inset, Fig 5.2). Since the metabolic demand was undoubtedly high, it might have been expected

that f_{sc} would remain elevated to meet the metabolic requirements, as it did during emersion. In contrast, even though there are large standard errors about the means, there is a clear pattern of a decrease in ventilation frequency over time following exercise in air. Such a pattern is more reminiscent of that occurring during recovery from exercise in water (Chapter 3; inset, Fig. 5.2), or in crabs in which breathing in air is the norm (eg *Birgus latro*, Smatresk and Cameron, 1981; *Cardisoma carnifex*, Wood and Randall, 1981b; *Holthuisana transversa*, Greenaway et al., 1983). However, in the air-breathers the oxygen supply was reduced as the requirement for oxygen decreased post-exercise. In *Jasus* $\dot{M}O_2$ was reduced to below the pre-exercise level after exercise, so a reduction in the supply of oxygen, as implied by the gradual decline in f_{sc}, is unexpected. Certainly, an internal hypoxia must have developed during exercise, and since $\dot{M}O_2$ was depressed throughout the air-exposed, post-exercise period, P_aO_2 must also have remained depressed over that time. Such an internal hypoxia would normally be expected to elevate the rate of scaphognathite beating (Taylor, 1987). A strong response of the scaphognathites was only observed during the initial stages after cessation of exercise, implying that the progressive reduction in f_{sc} is related to factors other than a low internal PO_2, and may perhaps be related to the breakdown of acid-base regulation or to excess lactate accumulation in the muscles or haemolymph of the exercised and air-exposed animals.

The response of heart rate to exercise in air also appears paradoxical, f_H falling to about two-thirds of the aquatic, pre-exercise value. This bradycardia is the reverse of that observed after exercise of *Jasus* in water (inset, Fig. 5.3), in vertebrates, and in most crustaceans, including the air-breathers *Cardisoma* and *Birgus* (Wood and Randall, 1981b; Smatresk and Cameron, 1981) and the water-breathers *Cancer* and *Callinectes* (McMahon et al., 1979; Booth

et al., 1982). However, Herreid et al. (1979) reported a similar bradycardia after exercise in the land crab, *Cardisoma guanhumi*, and suggested that it was a response to low internal PO_2. Hypoxia is reported as causing bradycardia in a number of crustaceans (McMahon and Wilkens, 1975; Taylor et al., 1977b; Wheatly and Taylor, 1981), and is thought to have effect heart rate through a reduction in P_aO_2 detected by peripheral and/or central chemoreceptors (Bush et al., 1987; Massabuau and Burtin, 1987; Taylor, 1987).

However, while heart rate later increased in both *C. guanhumi* and *Jasus*, it is important to note that *Cardisoma* was in its natural respiratory environment. If low internal PO_2 was the factor responsible for the bradycardia, then this stimulus would be removed by the crabs ability to restore haemolymph oxygen tension. *Jasus*, on the other hand was incapable of sustaining $\dot{M}O_2$ at any rate approaching that measured pre-exercise and presumably PO_2 was also low during the latter part of Stage I recovery. Thus it is probable that heart rate, like ventilation frequency, is controlled by factors other than, or in addition to, PO_2.

In addition to the 7 $mmol.l^{-1}$ rise in haemolymph [lactate], emersion following exercise also produced a significant and continuous rise in haemolymph PCO_2 (7 Torr over the 8h) the most rapid rate of increase occurring between 0-1h post-exercise.

Both metabolic and respiratory acids act to depress haemolymph pH. By comparing the pH changes with those recorded during recovery from exercise in water and those during emersion (Figs. 3.5 and 4.4, also inset, Fig. 5.4), it appears as though the post-exercise period in air can be divided into two stages. The first stage appears to be the result of exercise alone, since in both exercise groups the pH changes between 0-1h post-exercise were almost identical (7.52 to 7.22 post-exercise in air, 7.53 to 7.18 post-exercise in water). The second part of recovery (1-8h) more closely resembles the pH changes

during emersion. Such a strict division of the causative effectors is unrealistic, however, since emersion also produced a significant drop in pH between 0-1h from 7.67 to 7.56. Thus if the effects of exercise and emersion on acid-base state are additive, then a larger pH change might have been expected, at least during the first hour. Moreover, the increases in PCO_2 and [lactate] were even greater in air post-exercise than the sum of these variables during aquatic exercise and emersion (insets, Figs. 5.5 and 5.7), suggesting that the acidosis was less severe than expected. This implies that some degree of buffering over and above that observed during emersion alone was operational, at least within the first hour post-exercise.

Animals which are held in air lack access to a readily available source of ions from sea water. There are, nevertheless, other routes which can be used in the regulation of acid-base status. Work on compensatory mechanisms in crabs during emersion has suggested that mobilising calcium carbonate could increase the buffering power of the haemolymph (Henry et al., 1981; deFur et al., 1980; Innes et al., 1986). While not significant, there was a small rise in calcium throughout the post-exercise, emersed phase which, if the mean values are considered, would contribute approximately 1.6 $meq.l^{-1}$ of bicarbonate to the buffer pool after 1h. That bicarbonate from some source was added to the haemolymph is shown by the maintenance of the bicarbonate concentration during the first hour in air. This happened at a time when acidification of the haemolymph by the addition of metabolic hydrogen ions might have been expected to reduce its concentration.

Additionally, ammonia has been implicated as having a role in acid-base balance. It is likely that ammonia is released as NH_3 rather than NH_4^+ (Mangum and Towle, 1977) and would therefore act as a proton sink. Measurements of ammonia concentration revealed an increase throughout the post-exercise, emersed period, probably

through additional protein catabolism and/or reduced ammonia excretion. The total change was small, however, at about 0.7 $mmol.l^{-1}$, and would have contributed little to reducing the acidity of the haemolymph.

Increases of both PCO_2 and of metabolic acids contributed to the progressive depression of haemolymph pH. Comparison of the calculated metabolic acid load ($\Delta H+_m$) with the change in lactate concentration (ΔLa^-) (Fig. 5.11) shows a close matching of the two variables up to 4h post-exercise, but at 8h post-exercise the lactate concentration was about 2.5 $meq.l^{-1}$ higher than that of ΔH^+_m. It is normally assumed that there is a 1:1 stoichiometry between lactate and hydrogen ions, regardless of their source (e.g. Portner et al., 1984). This discrepancy implies that between 4-8h lactate moved from the tissues into the haemolymph independently of H^+ ions or that additional buffers were added to the blood. If the latter occurred, the source of the buffer is unclear. There was essentially no change in the rate of production of calcium over that previously observed. Ammonia appeared at a slightly higher rate between 4-8h than earlier into emersion, but the total change was much less than the 2.5 $meq.l^{-1}$ metabolic acid deficit. Smatresk et al. (1979) and Smatresk and Cameron (1981) have also reported similar discrepancies between ΔH^+_m and ΔLa^- during recovery from exercise in the land crabs *Gecarcinus* and *Birgus*, respectively. It is possible that some of the hydrogen ions associated with lactate were retained intracellularly or that Na^+/H^+ or Cl^-/HCO_3^- countertransport in effect returned protons to the intracellular compartments.

Returning *Jasus* to water (Stage II recovery) produced a rapid increase in oxygen consumption, although the peak rate did not occur until the animals had been in water for an hour. When compared to the pattern observed during recovery from exercise in water (Chapter 3), $\dot{M}O_2$ remained elevated for considerably longer in lobsters exercised

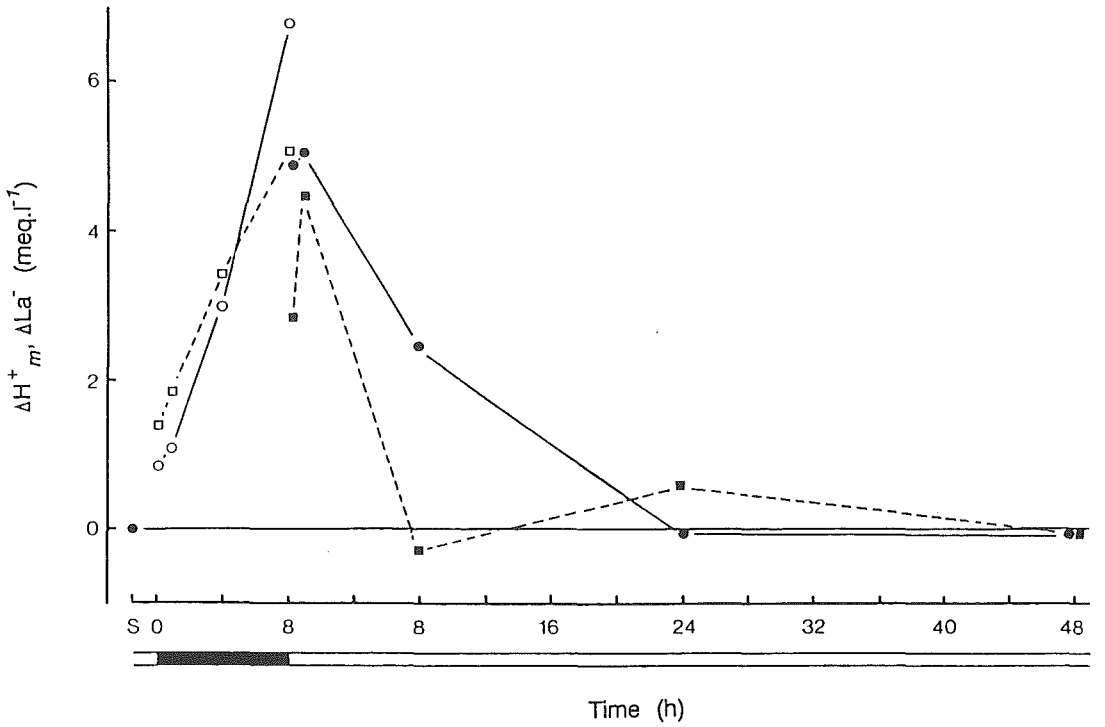

Fig. 5.11 Mean changes in the calculated metabolic acid load (ΔH^+_m □■ — —) and lactate load (ΔLa^- ○● ——) during emersion following exercise and during subsequent reimmersion. By definition, the value of both variables is equal to zero in settled, submerged animals (S). Other details as described in Fig. 5.2.

in air, only returning to the pre-exercise rate after 48h reimmersion. This is consistent with a greater O_2 debt associated with emersion after exercise. Partitioning of the oxygen debt into lactic and alactic portions is common in vertebrate physiology (Bennett, 1978) and it may also be applied to crustacean studies. Fig. 5.12 illustrates the total oxygen debt estimated from the oxygen consumption data given in Fig. 5.1, and the lactate component of the O_2 debt as calculated in Chapter 3. This calculation is based on the assumption that lactate is distributed throughout the body fluids at the same concentration as that measured in the haemolymph.

The total oxygen debt resulting from emersion after exercise was large (15.6 mmol $O_2.kg^{-1}$, Fig. 5.12). Sufficient oxygen was used over the 48h recovery period to fuel the resting rate of metabolism for nearly 20h. This value is extremely high when compared to that of air-breathing crustaceans. From the data of Herreid et al. (1979), exercise produced an estimated O_2 debt of only ~3 mmol $O_2.kg^{-1}$ in *Cardisoma guanhumi*, which is similar to that found by Wood and Randall (1981a) in *C. carnifex* after mild exercise. The latter authors also reported that severe exercise of *C. carnifex* produced an O_2 debt of ~7.4 mmol $O_2.kg^{-1}$, which is still less than half the total debt incurred by *Jasus*. A major difference exists between *Jasus* and *Cardisoma* spp., in that the crab species are land-dwelling, and as such are able to elevate oxygen consumption both during and immediately after exercise in air. By contrast, $\dot{M}O_2$ in *Jasus* did not rise post-exercise, and was even depressed compared to the aquatic rate. Since the resting level of oxygen consumption was not sustained, *Jasus* has the problem of regaining the oxygen 'lost' during the post-exercise period in air (~6 mmol $O_2.kg^{-1}$).

Part of the oxygen used in reimmersion was required to remove lactate (which is apparently not excreted in crustaceans, Bridges and Brand, 1980). While the lactic portion of the debt itself was

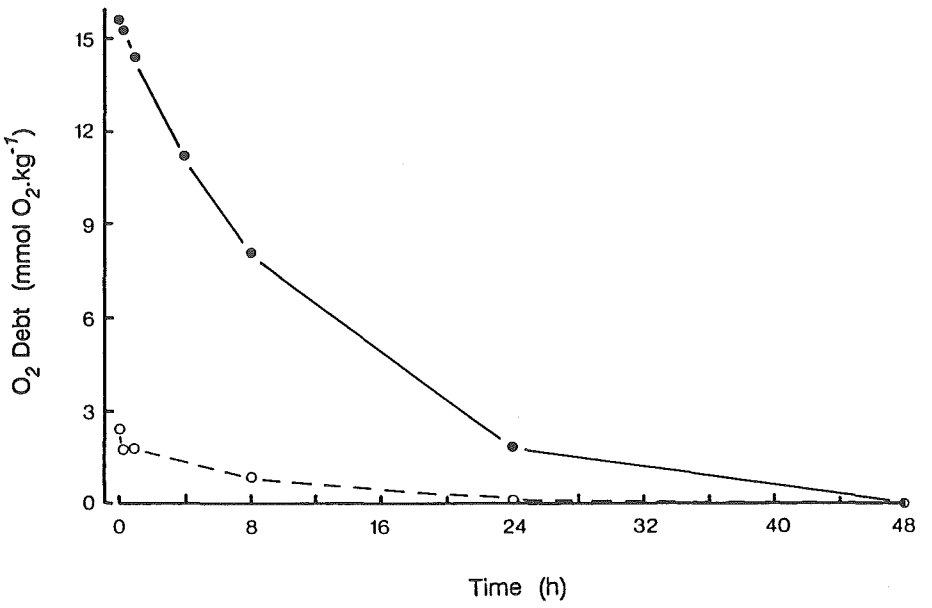

Fig. 5.12 Changes in the oxygen debt (● ——— ●) and the estimated lactate component (o — — —-o) during recovery from the combined effects of exercise and emersion. The O_2 debt was calculated from the data given in Fig. 5.1, and the lactate component from the data in Fig. 5.7. Note the considerable quantitative discrepancy between the two variables.

reasonably large, it was only about 16% of the total debt present immediately on re-entry into water, and remained at approximately the same proportion of the total debt until it was removed (Fig. 5.12). As has been suggested previously, some of the lactate (and associated hydrogen ions) may have been retained within the cells, resulting in an underestimate of the metabolic cost of removing lactate.

Part of the oxygen debt in rock lobsters held in air after exercise must related to the fact that $\dot{M}O_2$ did not increase as it did following exercise in water. However, even though $\dot{M}O_2$ increased after 50 tail-flips in water, there was still a debt of ~4 mmol $O_2.kg^{-1}$ (Chapter 3). An 'exercise debt', of a similar magnitude to that observed during exercise and recovery in water, would be expected to result from exercise and recovery in air. Since the oxygen was obviously not taken up during the emersion phase, compensation was delayed until the animals were returned to the medium in which efficient oxygen uptake was possible.

Returning *Jasus* to water also produced increases in the rate of beating of the scaphognathites and heart. Although the frequencies of both the heart and scaphognathites rose rapidly on re-entry into water, there was some delay before either f_{sc} or f_H increased to its maximum. The peak in f_{sc} occurred at 2h while that of f_H was 6h later at 8h into the recovery period. The slow response of both organs is somewhat surprising. There would certainly be a high O_2 demand by the exercised tissues, so presumably the most beneficial response would be to maximise gas exchange as rapidly as possible on return to water, enhancing oxygen uptake as well as promoting excretion of CO_2 and other products accumulated during the previous 8h emersion. While $\dot{M}O_2$ peaked at 1h reimmersion the scaphognathites and the heart were not yet pumping at their maximum rates. The lack of correlation between $\dot{M}O_2$ and the heart and scaphognathites may again be the result of lactacidosis or fatigue of the muscles controlling the heart and

scaphognathites. However, it indicates that factors other than ventilation and perfusion were controlling gas exchange. Variations in cardiac output, such as those reported to occur in other crustaceans after exercise (McMahon et al., 1979: Wood and Randall, 1981b), could provide a better matching of perfusion to ventilation which would improve oxygen uptake. In addition, greater extraction of oxygen from the inspired water would also result in the observed increase in $\dot{M}O_2$.

Increased ventilatory and cardiac frequencies would also serve to enhance the CO_2 gradient between the animal and the external medium. Within an hour of reimmersion calculated PCO_2 fell by 50% to 4.3 Torr. Haemolymph pH did not rise as rapidly as expected given the PCO_2 change, since it was accompanied by a metabolic acidosis which lowered $[HCO_3^-]$ simultaneously. The pH increase during the first hour of reimmersion was only about 0.2 units. A similar reduction in bicarbonate concentration was observed after exercise in water (Chapter 5; and inset, Fig. 5.6) and in the crab *Callinectes* during and after exercise (Booth et al., 1984). While a reduction in $[HCO_3^-]$ seems contrary to the acid-base requirements of the animal, the decrease in HCO_3^- also represents a loss of H^+ from the haemolymph.

At eight hours into reimmersion f_{sc} and f_H were still elevated and the calculated CO_2 tension continued to decline slowly. PCO_2 at this time was significantly lower than the aquatic, pre-exercise value. This respiratory alkalosis at 8h, acting in concert with a very small metabolic alkalosis, resulted in mean haemolymph pH being raised slightly (although not significantly) over that measured in the controls.

Between 0-8h reimmersion ΔH^+_m decreased more rapidly than ΔLa^-, implying that the excretion or elimination of acid occurs faster than lactate. Nevertheless, remetabolism of lactate must consume a similar quantity of hydrogen ions. The balance of the decrease in ΔH^+_m

between 1-8h may be therefore be explained by H^+ excretion, possibly in exchange for Na^+ or with an anion such as chloride.

By 8h into reimmersion, [lactate] was still more than 2 $mmol.l^{-1}$ higher than at rest, but the metabolic acid load in the haemolymph was completely eliminated. Thus the restoration of lactate to the pre-exercise level by 24h reimmersion probably involved selective uptake of the hydrogen ions required to metabolise lactate from the surrounding sea water. Both *Callinectes* (Booth et al., 1984) and the trout *Salmo gairdneri* (Holeton et al., 1983) also showed a similar persistence in lactate load despite reduction of ΔH^+_m to zero. These authors have also concluded that hydrogen ions are excreted to protect acid-base status, removal of lactate apparently being of secondary consideration.

Excretion of ammonia could also be used as an alternative or supplementary mechanism for removal of hydrogen ions. For ammonia excretion to be effective in reducing $[H^+]$ it would have to be produced in the tissues as NH_3 and excreted as NH_4^+. Reimmersion produced a rapid clearance of ammonia from the haemolymph. Within an hour it had returned to approximately the same concentration as in the haemolymph of the pre-exercise controls. Its rapid decrease is probably also a reflection of the higher ventilatory and circulatory activities which would maintain a larger gradient across the gills for ammonia excretion. The total change was, however, quite small and at most would have produced a reduction in $\Delta H+_m$ of only around 0.6 $mmol.l^{-1}$.

In summary, *Jasus* appears unable to fully compensate the effects imposed by deprivation of water after exercise. Again, a major factor influencing the response appears to be the effective loss of the gills, which not only prevented effective gas transfer, but also removed a major route for acid-base compensation. However, while the acidosis was greater than that observed during either emersion alone

or following immersed exercise, it was less than the sum of the two individual treatments, which suggests that *Jasus* may be able to elevate the concentration of blood buffers above that during air exposure.

CHAPTER 6

VARIATIONS IN SEA WATER QUALITY FROM A COMMERCIAL SYSTEM AND ITS EFFECT ON SOME HAEMOLYMPH VARIABLES IN *JASUS EDWARDSII*

ABSTRACT

A study was made to assess possible causes of mortality of *Jasus* held in a commercial, recirculating seawater system. The system consisted of up to 50 rock lobster tanks, arranged vertically in stacks of 5 tanks. Up to 8 animals were put into each tank. The system capacity was therefore 400 rock lobsters. Measurements were made over two pre-export holding periods ('runs') on animals and sea water from a single stack within the system. Changes in seawater variables were assessed at different levels of the system from the inflow, through the uppermost tank (Tier 1) to the lowest level (Tier 5), and from the filter. Daily measurements of PO_2, pH, titration alkalinity (TA), Cl^- and ammonia concentrations were made during each run. Sea water data were pooled either vertically, showing the changes within different levels of the system, or on a daily basis, reflecting overall trends within the system. Pre-branchial haemolymph was assayed for ammonia, total CO_2 content (CCO_2) and lactate, samples being removed from 4 groups of animals, including: (1) controls in flowing sea water; (2) rock lobsters landed at the factory; (3) animals from the commercial system; and (4) 'recovery' specimens which were removed from the recirculating system and transferred to flowing sea water.

Blood sampled from air-exposed rock lobsters showed high levels of ammonia (2.02 $mmol.l^{-1}$), lactate (6.61 $mmol.l^{-1}$) and CCO_2 (7.3 $mmol.l^{-1}$) compared to the same variables measured im lobsters held in

flowing sea water. Transferring the animals to the recirculating system produced rapid reductions in both haemolymph [lactate] and [ammonia] within the first 24h. Blood lactate remained at about the control level throughout the runs, but haemolymph ammonia remained high at about twice the control level. Total CO_2 took longer to decrease and reached a similar level to that measured in the controls only after 5 days in the system.

There was relatively little change in any of the seawater variables from the inflow down to the lowest animal tank, although there were slight tendencies for PO_2 and pH to decrease, and NH_3 and TA to increase, from the inflow to the lowest level. It was concluded that these differences would be unlikely to cause more stress to the animals at the lower levels.

When the changes were assessed on a daily basis it was found that there was little change in PO_2 or $[Cl^-]$ over either run, but there were marked changes in the other seawater variables. Both titration alkalinity and ammonia concentration continually increased over the first half of Run 2, TA rising by about 0.5 $mmol.l^{-1}$ over 5 days and ammonia increasing from virtually zero to 0.28 $mmol.l^{-1}$ over 4 days. The increase in NH_3, and therefore TA, reflects dumping of ammonia by the animals and decomposition of faecal material and dead animals beyond the capacity of the filter to remove it. The pH of the commercial system's water was always lower than that of fresh sea water (pH of fresh sea water = 8.03), and probably resulted from CO_2 output by the rock lobsters in the system. In both runs seawater pH decreased when the animals were first introduced to the system, and within 1 - 2 days had stabilised at around 7.1 - 7.2 units in Run 1 and 7.4 - 7.5 in Run 2.

Moribund animals transferred to flowing sea water showed few signs of recovery. Concentrations of the CCO_2 and ammonia were not restored to the control levels and the rock lobsters remained

listless and unresponsive to handling. It is concluded that the recirculating system's water oxygenation and temperature control were satisfactory and that, on the basis of the variables measured, seawater acidity and ammonia content were probably the eventual causes of the high mortality. Conclusions and recommendations are made for handling and treatment of the animals to ensure maximal survival.

INTRODUCTION

In recent years commercial fishing enterprises have diversified from merely processing rock lobsters for the local and overseas market to the more lucrative market of exporting the animals live. Inherent in this practice is the development of a system capable of holding several hundred animals for up to several weeks before they are packaged and flown overseas. Setting up a new venture entails finding the best compromise between ensuring the animals are kept healthy and undamaged in order to fetch the best possible price in a competitive marketplace , and maintaining the lowest possible operating costs.

Live crabs and lobsters are regularly shipped from coastal regions of the British Isles to inland markets and the European continent. In the velvet crab, *Liocarcinus puber*, and the brown crab, *Cancer pagurus*, problems with high mortality were encountered from first capture of the animals to their arrival in Spain (Whyman et al, 1985 and Uglow et al, 1986, respectively). The authors of both reports concluded that handling procedures, especially air exposure, and poor water quality, particularly oxygenation and temperature control, were major contributors to the death of the animals. They also pointed out that the conditions used for handling and transport

of a successfully exported species were not necessarily suitable for shipment of others.

The effects of air exposure on respiration and haemolymph chemistry may vary in magnitude between species but generally results in a reduction in oxygen uptake and accumulation of anaerobic metabolites in the blood leading to a haemolymph acidosis (Chapter 4, Taylor and Wheatly, 1980, 1981; Taylor and Whiteley, 1989). Transferring an aquatic animal back to water may alleviate these initial effects but, depending on the system used, may produce additional problems for the animals if the water quality is poor.

Several systems may be used to store crustaceans before shipment. A report produced by Ayres and Wood (1977) details some of the systems used to hold lobsters for live export, including holding facilities based on tidal exchange of sea water, flow-through systems where fresh sea water is pumped to the lobsters before running to waste, and recirculating units where the water is filtered and recycled throughout the course of the holding period. The venture discussed here used this last system, but found loss of condition and high mortality during storage of the rock lobsters. A study was made over two pre-export holding periods (runs) to assess the condition of the sea water and of rock lobsters held in the system and to predict the possible causes of the death of the animals prior to shipment. Understanding the changes occurring in the system as a whole not only highlights the present problems but would also point to potentially better methods of holding the animals and ultimately ensuring their higher survivorship.

MATERIALS AND METHODS

During May and June, 1986 a study was made to examine possible causes of the high mortality of rock lobsters intended for live export at a commercial enterprise in Kaikoura. Rock lobsters are normally held in a recirculating sea water system (approximate capacity = 5000l) for about one week after capture to allow them to recover from capture and handling and achieve a condition suitable for safe air freight. Sea water was pumped from a large reservoir in the basement up to the live animal room where it was gravity-fed through the tanks and then returned to the basement (Fig. 6.1). Aeration was provided by this cascading of water through the system and, during Run 2, was augmented by pumping air through the reservoir. For a normal run the air is cooled in the rock lobster room and the sea water initially maintained at about 10°C until about 2-3 days before export, when the water in the animal room is isolated from the rest of the system and the temperature brought down to about 6°C (G. Harmon, pers. comm.).

Measurements were made for 2 holding periods: Run 1, 12-17 May: Run 2, 29 May - 5 June. The most complete set of data are available for Run 2 and unless otherwise stated, observations refer to this run. During Run 1 approximately one third of the water in each circuit passed through a shell filter. However, before Run 2 the filter system was renewed and enlarged and the circulation changed so that all of the water passed through the filter on each circuit.

The system is designed to hold up to 400 rock lobsters. Ten stacks of tanks are held in the animal room with 5 tiers in each stack. Up to 8 animals may be put into each tank, which contained an estimated 25 - 30 litres of sea water. At the start of Run 2 the sea water was changed and a total of about 320 freshly caught animals were added to the system over the first 2 days. All sea water and

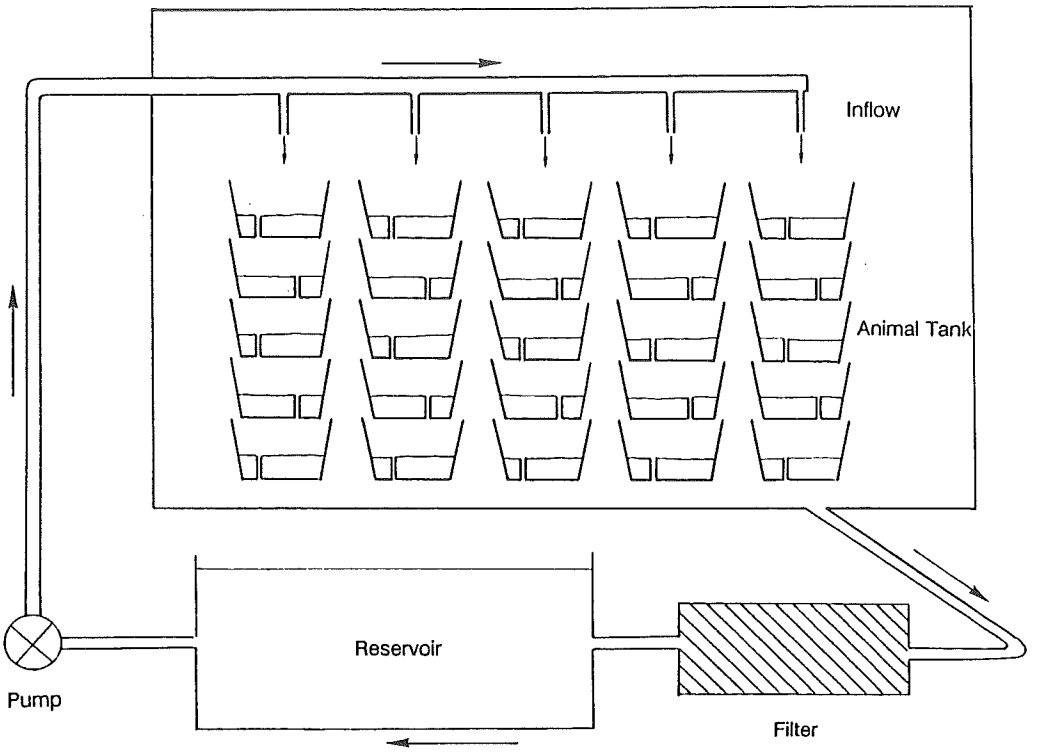

Fig. 6.1 Diagrammatic representation of a recirculating system used to hold rock lobsters, *Jasus edwardsii*, prior to live export. Each animal tank may contain up to 8 rock lobsters. The direction of water flow is indicated by the arrows. An additional 5 stacks of tanks are not shown. Further details in the text.

blood samples were removed from a single stack of tanks. Run 1 did not represent a typical situation, since the system had been filled to capacity one week previously. One stack of 40 animals was replaced with freshly caught ones on the day that the measurements were commenced and all observations were made on this stack. The water conditions, however, would have been dominated by those pre-existing in the system.

During Run 1 sea water was sampled from the inflow channel which supplied water to the stack, from each of the 5 animal tanks (Tier 1 = top, Tier 5 = bottom) and from the filter outlet. Daily blood samples were taken from a single rock lobster from each tier. In Run 2 seawater samples were taken from the inflow, from Tiers 1, 3 and 5 only, and from the filter. Blood was also sampled daily from 3 animals in each of Tiers 1, 3 and 5.

The animals held in the commercial system were compared with several control animals held in flowing sea water at the George Knox Research Facility. Blood was also sampled from rock lobsters which had been in air for several hours, which is the condition of the animals on entering the system after capture. In Run 2 unacceptably high losses had occurred on the morning of Tuesday 3rd of June and all of the rock lobsters were lethargic with low muscle tone. Following seawater and haemolymph sampling, all animals except those being examined were removed from the system, and temperature control removed. Four of these remaining animals were later taken to the research laboratory to assess recovery in flowing sea water.

Seawater samples were analyzed for pH, oxygen tension (PO_2), ammonia and chloride concentrations. Titration alkalinity was also measured during Run 2. The temperature of the system was recorded daily. Haemolymph samples were analyzed for ammonia, total CO_2 (CCO_2) and lactate concentration. It was, unfortunately, not possible to determine haemolymph pH since the blood samples rapidly clotted, and

insufficient blood was able to be obtained for analysis.

Analytical Methods

Sea water pH was determined using a portable pH meter (Metrohm) calibrated with IL buffers (pH = 4 and 7). Oxygen tension was determined with a IL O_2 electrode connected to a Strathkelvin meter or, more infrequently, with a hand-held portable pH/O_2 meter (Hanna, HI 8114). Calibration was made using a zero O_2 solution and air. Sea water titration alkalinity (TA) was determined using the potentiometric titration method of Almgren and Fonselius (1976), and involved titrating 100 ml samples with 0.01N HCl. Sea water chloride concentrations were measured on 20 μl samples with a Corning EEl Chloride Meter.

Pre-branchial blood samples were removed anaerobically from the base of the walking legs via the arthrodial membrane using 20 gauge needles attached to 1 ml plastic syringes. Both sea water and blood ammonia content were determined with a Boehringer test-combination kit for urea. Sea water ammonia was measured on 100 μl samples while the amount present in pre-branchial haemolymph was determined on 20 μl samples. The change in absorbance was measured at 640nm with a Pye Unicam SP1800 spectrophotometer.

Haemolymph CCO_2 was determined on 20 μl samples using the method of Cameron (1971). Each haemolymph sample was bracketed between 20 μl samples of 10 mmol.l^{-1} $NaHCO_3$ standards.

Blood lactate concentrations were measured on 50-150 μl samples using a Boehringer food analysis kit for lactate, in which the glycylglycine buffer had been modified by the addition of 12 mmol.l^{-1} EDTA as a chelating agent for Cu^{++} (Engel and Jones, 1978).

All haemolymph analyses were performed at the George Knox Research Facility on samples which had been removed anaerobically and stored briefly on ice before analysis (~10 min.).

RESULTS

Composition of Normal Sea Water

Samples were taken from the sea water supply to the George Knox Research Station, which is taken from about 100 m offshore. At 15°C and saturated with air it had a mean pH of 8.03 and a titration alkalinity (TA) of 2.35 $mmol.l^{-1}$. Ammonia was virtually undetectable (<0.01 $mmol.l^{-1}$).

Vertical Differences in Seawater Composition within the Stack

Pooling the daily data for each level permits examination of differences in seawater composition at different levels in the stack due to metabolic exchanges with the animals. Exchanges of gases with the atmosphere would tend to reduce these effects and in fact they were quite small. In Run 2 PO_2 tended to decrease slightly down the stack (Fig. 6.2), dropping from around 145 Torr in the inflow to about 129 Torr in the lowest tank (Tier 5). However, even in the lowest tanks PO_2 was usually about 85 - 90% of inflow PO_2, and never dropped below 110 Torr, or about 80% of inflow PO_2. A similar pattern was also seen in Run 1 (Fig. 6.2), but on May 15 the oxygen tension in Tiers 4 and 5 dropped to about 60% of the inflow. The inflow was essentially air-saturated at all times (140-150 Torr).

Likewise, pH showed a progressive, albeit small, decrease from the inflow to Tier 5, dropping from a mean inflow pH of 7.56 ± 0.05 to 7.46 ± 0.05 in Tier 5. Sea water emerging from the filter had a similar pH to that of the inflow.

Titration alkalinity was not attempted for each of the sampling points. The data suggest a very small (<1%) increase in TA from the inflow to Tier 3. The TA of the filter water was generally between those of the inflow and Tier 3, which is consistent with the removal of ammonia.

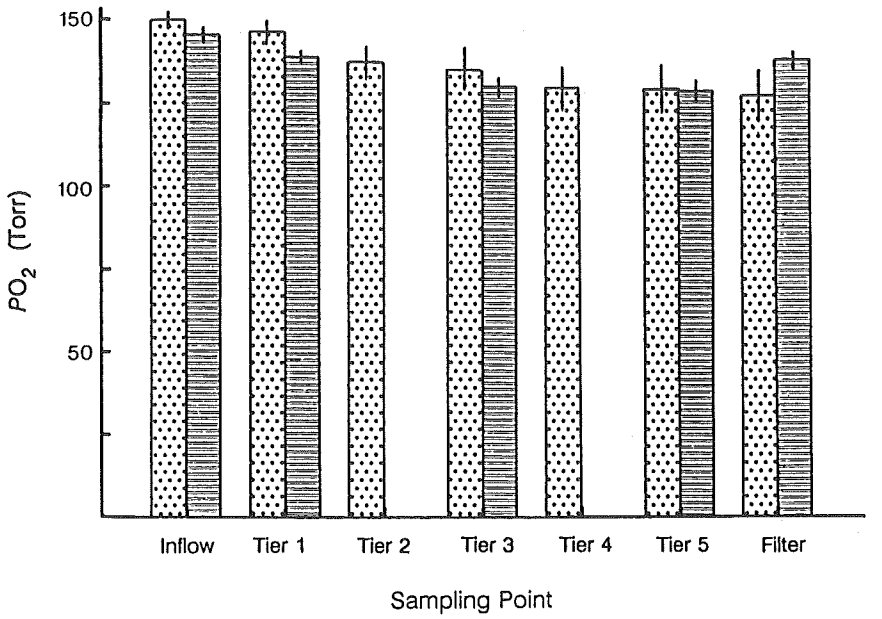

Fig. 6.2 Mean vertical changes in sea water PO_2 at different sampling sites in a closed, recirculating seawater system. Values (mean ± 1 SEM) were obtained by pooling daily data for each level. Tier 1 = top, Tier 5 = bottom. Temperature ~10°C.

= Run 1, = Run 2.

Ammonia levels in the rock lobster tanks were generally higher than in the inflow water by about 0.02 - 0.04 $mmol.l^{-1}$. These differences were insignificant compared to the overall level of ammonia in the system and the large daily fluctuations which were observed. Levels in the filter were similar to the inflow values, indicating its removal by the filter.

Daily Changes in Seawater Composition

Figs. 6.3-6.6 illustrate the daily changes in the composition of the sea water in the tanks holding the animals (the mean of Tiers 1, 3 and 5) during Run 2. As noted above, differences between different parts of the system at any one time were small, so these trends reflect those of the system as a whole.

There was little change in the temperature of the animal room over Run 2. The air was cooled rapidly and over the majority of the run water temperature was about 9.5°C. On the last 2 days temperature control was removed and the water temperature rose by 3°C.

Titration alkalinity (TA, Fig. 6.3) was not measured before the animals were added to the system for Run 2. However, the water had been changed and would have been similar to normal sea water (about 2.35 $mmol.l^{-1}$). TA rose rapidly, peaked after 4 days at 2.83 ± 0.01 $mmol.l^{-1}$ and then fell again equally rapidly to a slightly elevated level after 7 days when the measurements were stopped.

Ammonia levels (Fig. 6.4) also rose sharply from virtually zero initially and followed a rather similar time course to that of TA, peaking at 0.28 $mmol.l^{-1}$ after 2 - 3 days and then falling to a somewhat raised level (0.07 $mmol.l^{-1}$) at the end of the run. The fall in ammonia levels indicates that the shell filter functioned to remove ammonia, although the capacity of the filter was initially exceeded. Ammonia was only measured on the final day of Run 1. Mean concentration in the tank was 0.06 ± 0.002 $mmol.l^{-1}$, similar to the

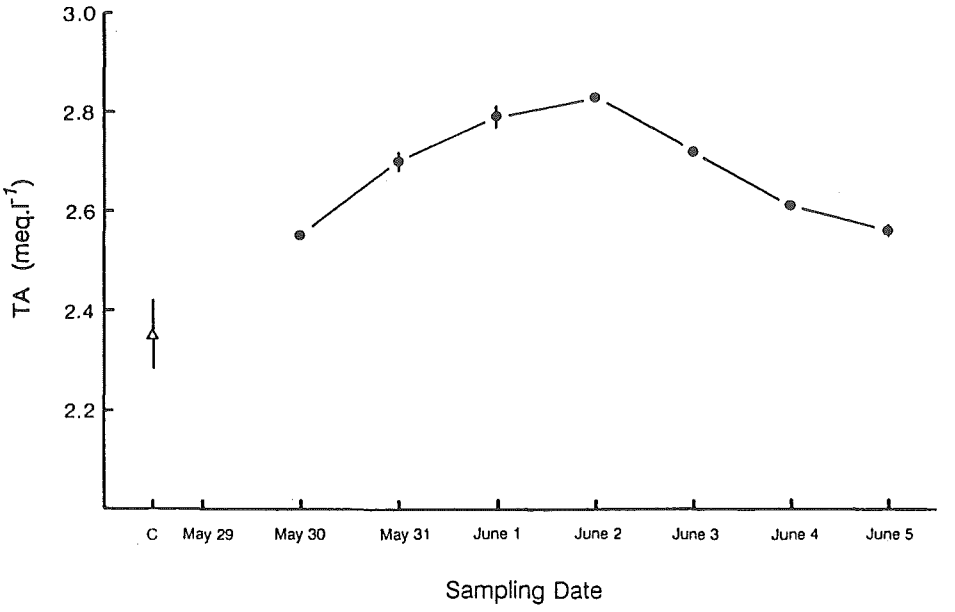

Fig. 6.3 Daily changes in mean titration alkalinity (TA) of sea water from a recirculating system during Run 2. Values were obtained by pooling data from different levels for each day. C = value in fresh sea water. Where error bars are not shown they lie within the limits of the symbol. T ~ 10°C.

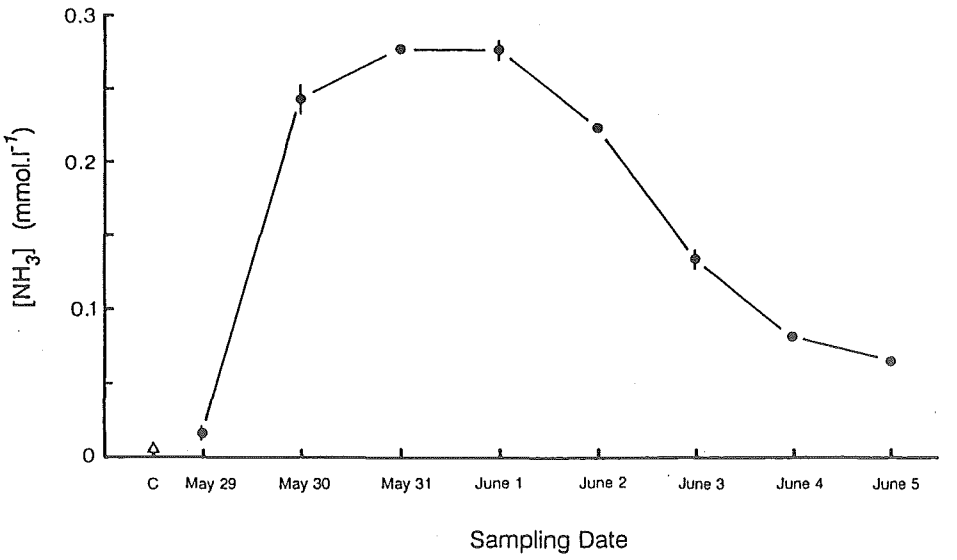

Fig. 6.4 Changes in mean daily ammonia content (= NH_3 + NH_4^+) in sea water from a recirculating system during Run 2. The value in fresh sea water (C) is denoted by the △. Other details as in Fig. 6.3.

value determined at the end of Run 2.

The pH of the seawater system (Fig. 6.5) was always lower than that of fresh sea water. During Run 2 the mean pH of the animal tanks rapidly fell to about 7.4 - 7.5 and then remained more or less constant. In Run 1 the pH was generally somewhat lower, falling to 7.1 - 7.2 in some of the animal tanks.

Mean daily PO_2 levels (Fig. 6.6) were relatively constant, varying between 120 - 140 Torr in the animal tanks, or about 90% of the inflow PO_2 during Run 2. As noted above, on one occasion during Run 1 oxygen saturation dropped to about 62% in the lower tanks, but in general oxygenation was clearly adequate.

Throughout Run 2 chloride concentrations varied little, ranging from a minimum of 540 $mmol.l^{-1}$ to a maximum of 562 $mmol.l^{-1}$, a difference of only 4%.

Blood Analyses

Blood samples were analyzed for lactate, total CO_2 and ammonia from 4 groups of rock lobsters:

1) Controls

These animals had been held in flowing sea water for several days at the George Knox Research Laboratory. The mean concentrations of the measured variables were: lactate, 0.16 $mmol.l^{-1}$; total CO_2, 3.08 $mmol.l^{-1}$; ammonia, 0.58 $mmol.l^{-1}$.

2) Rock Lobsters held in Air for Several Hours

This group indicates the condition of the animals just prior to being introduced to the system. There were high levels of all 3 variables measured. Total CO_2 content (7.3 ± 1.7 $mmol.l^{-1}$, Fig. 6.7) and ammonia concentration (2.02 ± 0.24 $mmol.l^{-1}$, Fig. 6.8) in the

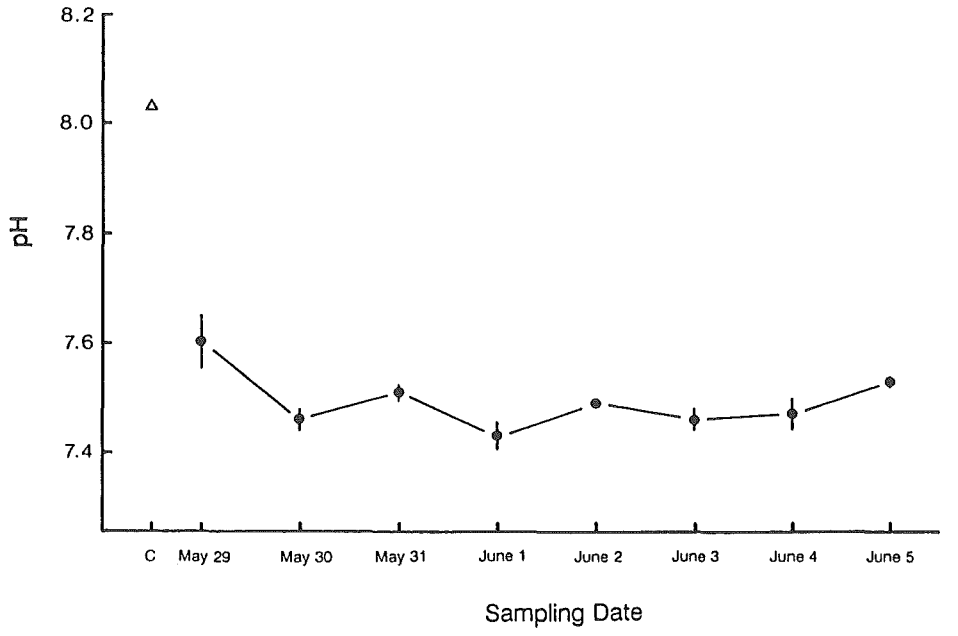

Fig. 6.5 Mean daily pH changes during Run 2. Details as in Fig. 6.3. Note the large difference in pH of the recirculated water compared to fresh sea water.

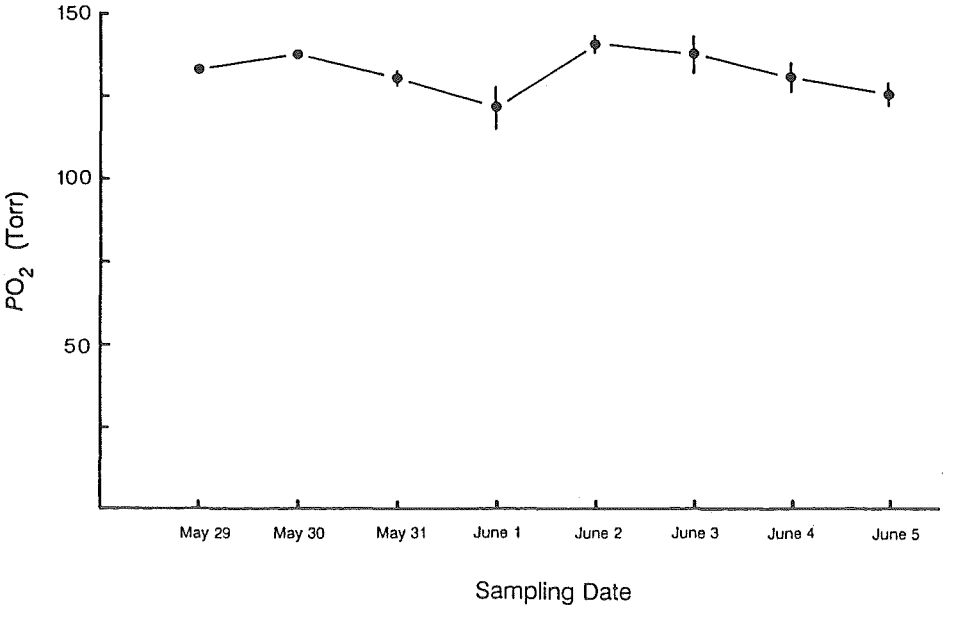

Fig. 6.6 Daily variations in seawater PO_2 during the course of Run 2. Further details as in Fig. 6.3.

pre-branchial blood of these aerial rock lobsters were 2 to 3 times higher than those in animals kept in the laboratory. The mean lactate concentration (6.61 ± 1.09 $mmol.l^{-1}$, Fig. 6.9) was some 40 times greater in the animals kept in air compared to those in flowing sea water. Lactate was highly variable and in some individuals was more than 11 $mmol.l^{-1}$.

3) Animals from the Recirculating Seawater System

As there was little variation between animals from successive tiers in any of the variables measured, the data have been pooled for each day. Fig. 6.7 shows that when the animals were put into the system total CO_2 in the blood was reduced, over a period of 4 days, to a level similar to that in the control animals. Blood ammonia concentration (Fig. 6.8), which was also very high in the air exposed rock lobsters, appeared to be reduced very rapidly to moderate levels when the animals were put into the holding tanks. A further small decrease occurred during the first 24 hours of their being in the system, the ammonia content then remaining relatively constant throughout the remainder of the run at approximately 1.1 $mmol.l^{-1}$, although this value was still about twice that of the control animals. The concentration of lactate in the blood was rapidly reduced from the high levels seen in the aerial animals when they were put into water (Fig. 6.9). Within 24 hours blood lactate was reduced to 0.26 ± 0.04 $mmol.l^{-1}$. It remained at about 0.2 $mmol.l^{-1}$ over most of the run, except on Day 6 when high levels in 2 animals raised the daily mean.

4) Animals Removed from the Recirculating System and Transferred to the George Knox Research Laboratory

Four rock lobsters which were in poor condition when Run 2 was terminated were transferred to running sea water for two days. At the

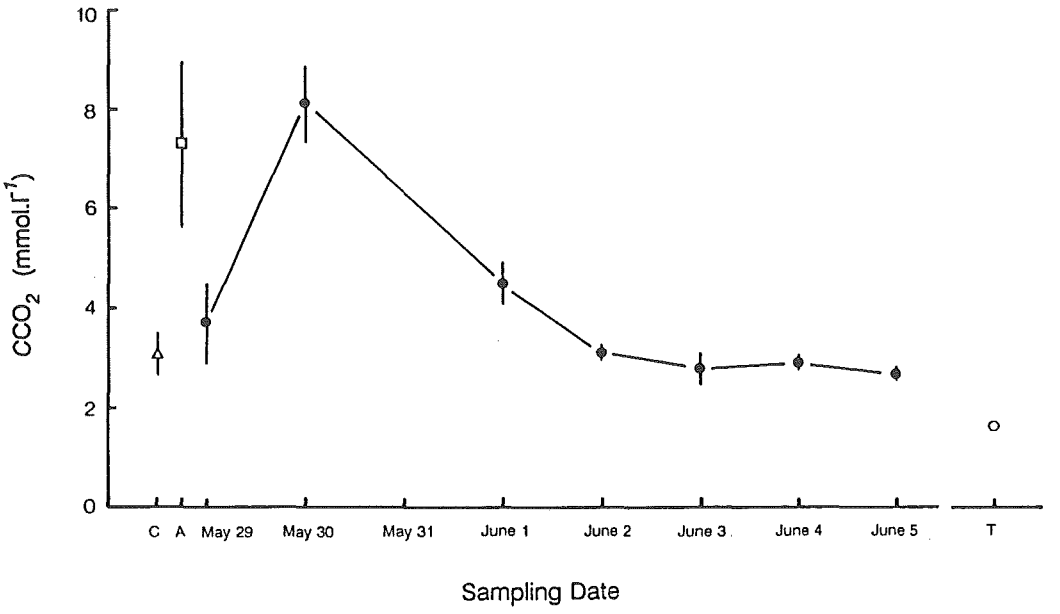

Fig. 6.7 Daily changes in blood total CO_2 (CCO_2) from rock lobsters held in recirculated sea water. Also shown are values determined for control animals held in fresh, flowing sea water (C, △), in rock lobsters emersed for several hours (A, □) and from moribund animals transferred from the recirculating system to fresh sea water (T, ○). Data are given as mean ± 1 SEM. Temperature ~10°C. Where error bars are not shown they lie within the symbol.

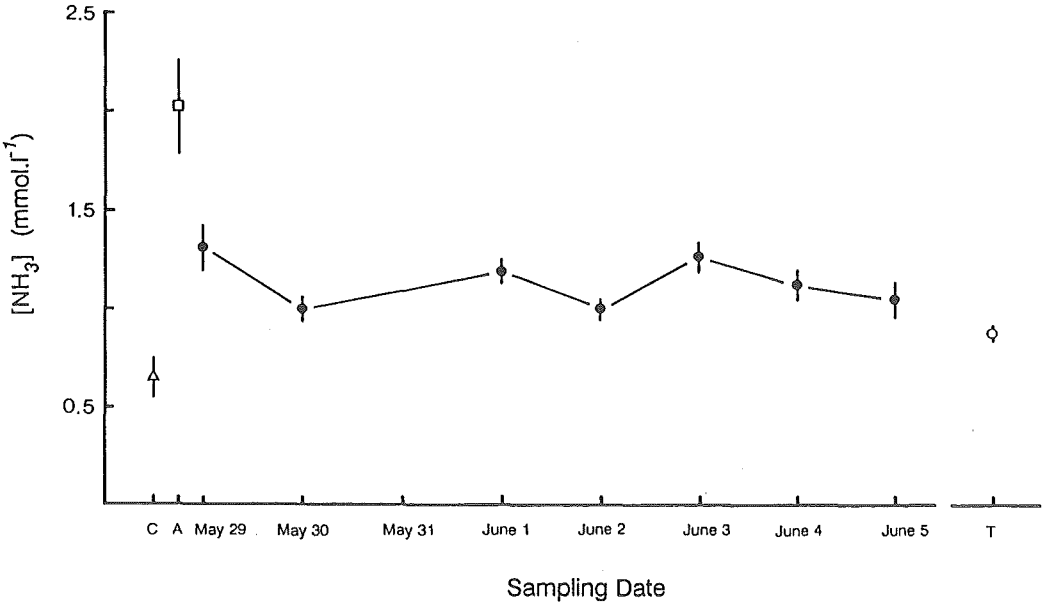

Fig. 6.8 Daily changes in ammonia content of haemolymph from animals in recirculating sea water. Also shown are control values (C, △), the concentration in emersed animals (A, □) and ammonia in animals transferred to fresh sea water (T ○). Temperature ~10°C.

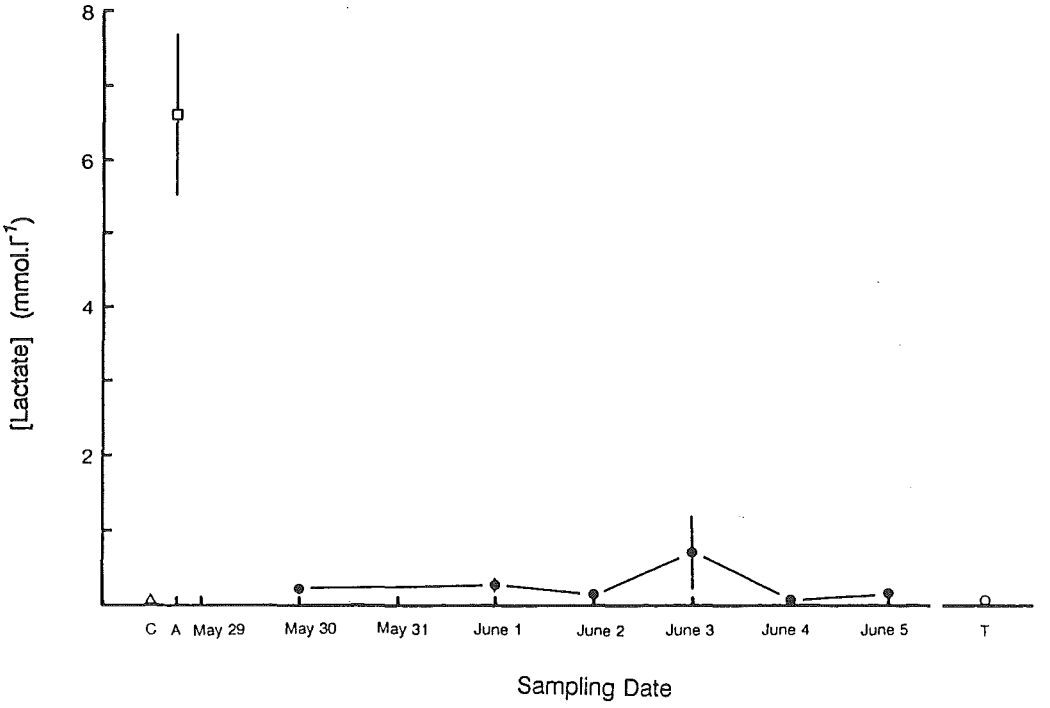

Fig. 6.9 Daily changes in haemolymph [lactate] in rock lobsters held in a closed, recirculating system. Other details as in Fig. 6.7.

end of the run lactate and total CO_2 were somewhat similar to control values while ammonia was somewhat elevated. During the 'recovery' period in flowing sea water lactate remained at about the same concentration as in the controls. However, total CO_2 (1.62 $mmol.l^{-1}$) actually dropped below control values and ammonia (0.88 $mmol.l^{-1}$) dropped a little but was still slightly higher than in the controls. The loss of condition was obviously not rapidly reversible since the rock lobsters remained listless and unresponsive to handling.

DISCUSSION

High levels of lactate and ammonia were measured in the blood of rock lobsters entering the factory after spending several hours in air. High levels of these substances would also undoubtedly be in their tissues. The build up of lactate indicates that the animals had switched to anaerobic metabolism, relating to the inability of the gills to function out of water and supply oxygen at the required rate. The build up of ammonia may also reflect the inability of the gills to effectively excrete it in air. Published reports on a number of crustaceans have shown that these substances, and more particularly, lactate, may increase steeply during exposure to air, although the quantitative values may vary considerably. For example, deFur and McMahon (1984b) reported a much larger increase in lactate than that measured here (9 - 14 $mmol.l^{-1}$), but the rise in blood ammonia was much smaller, changing by only 0.1 $mmol.l^{-1}$ compared to nearly 1.5 $mmol.l^{-1}$ increase recorded in freshly landed *Jasus*. Both of these substances are potentially quite toxic and the animals must be regarded as being very stressed at that time.

Production of lactate is normally associated with increased levels of hydrogen ions, leading to a metabolic acidosis. It is

therefore likely that the rock lobsters in air were also in severe acid-base disturbance. Ammonia, if released in the base form, NH_3, would offset the effects of acid to some extent. However, the molar concentration of lactate was about three times that of ammonia at that time, so that the net effect would be an acidosis, tending to lower the pH of the blood. In addition, the probable impairment of gas exchange at the gills implies that carbon dioxide would be accumulating, leading to a respiratory acidosis.

The rise in total CO_2 (essentially bicarbonate) which was seen in the blood of the air-exposed rock lobsters is indicative that some compensation for an acidosis was occurring, but since haemolymph pH was unable to be measured, it is not known whether the adjustment was complete. Comparison with the changes in total CO_2 and lactate determined during experimental emersion, and the changes measured in pH in those experiments (Chapter 4), indicates that the observed rise in total CO_2 would be insufficient to compensate for the presumably elevated concentration of hydrogen ions. The fact that the lactate concentration in the freshly landed rock lobsters was about 2 $mmol.l^{-1}$ higher than that induced experimentally, while the total CO_2 levels were about the same, suggests that haemolymph pH of the factory animals could be even lower than that measured at the end of 8h emersion (7.43 ± 0.03, Chapter 4), but probably higher than that measured at the end of an experimental, post-exercise period in air (Chapter 5).

On placing the animals into the seawater system a reversal of these effects was begun. However, recovery was complicated by the fact that the animals were in a closed recirculating system whose composition was affected by the activities of the animals, the operation of the filter, aeration, and rapid decomposition of animals that died.

Lactate is obviously cleared from the blood quite quickly,

probably within the first day, since blood lactate values of animals from the sea water system were generally comparable with those of control animals kept in running sea water. The initial period is a critical one for oxygenation of the system because the animals would be expected to have a high oxygen demand while they repay the oxygen debt associated with air exposure. In addition, they are likely to be more active (with a consequently higher oxygen consumption) during the early stages as they jostle for position with others in the tank. For aquatic animals there is a critical oxygen tension below which the oxygen supply becomes limiting. At 15°C in settled *Jasus* this level is around 80 Torr (Chapter 2); at higher metabolic rates such as those which almost certainly occur at least over the first 24 - 48h, the critical P_{O_2} is liable to be considerably higher. Using the $\dot{M}_{O_2}$ vs inspired P_{O_2} diagram in Chapter 2 (Fig. 2.7), extrapolating to a higher oxygen consumption, such as 20 $\mu mol.kg^{-1}.min^{-1}$, yields a critical P_{O_2} close to 100% saturation at 15°C. It appears that oxygenation was not a particular problem during the runs examined, although it was noted that on one occasion oxygen saturation decreased to about 60%, or close to the critical P_{O_2} of settled animals at 15°C (Chapter 2). Oxygen used by bacteria in the shell filter did not appear to reduce P_{O_2} significantly. Reducing the risk of oxygen depletion is vital, especially during the early stages. Eliminating uncontrolled temperature rises, which not only increases the metabolic rate but also reduces the oxygen content of the water, and bacterial blooms due to contamination with faeces or dead animals (the latter effect probably causing the depletion in animal numbers noted) is essential to maintain the oxygen supply to the animals. If these problems occurred, either singly or in combination, the effects could rapidly be disastrous.

An additional factor that could ultimately influence rock lobster survivorship is the concentration of nitrite (NO_2) within the

system. NO_2 is produced by the conversion of nitrogenous waste products by bacteria and is often regarded as one of the most toxic and critical factors influencing crustacean mortality (Malone and Manthe, 1985). In addition to well acclimated biofilters, good aeration is required to control its level. The data shown by Malone and Manthe indicated an increase in [nitrite] which occurred after the peak in [ammonia]. Thus, although not measured, NO_2 may have been a problem in the commercial system under investigation here.

An equally important reason for maintaining good aeration is that it removes carbon dioxide from the water. CO_2 is produced continuously during respiration at about the same rate as oxygen is consumed. As noted above, its conversion to carbonic acid leads to acidification both of the sea water and the blood. The fact that the pH of the sea water was at all times 0.5 to 1.0 units lower than fresh sea water (representing a 4 to 10-fold increase in the concentration of hydrogen ions) must be due to carbon dioxide. Other acids may have been excreted but since titration alkalinity did not decrease (and indeed, was actually increasing over the first four days) they are not quantitatively important or are neutralised by calcium carbonate in the shell filter. Reducing seawater pH to this extent is indicative of a 1 to 4 Torr rise in PCO_2 (determined from the data of Dejours, 1981). In studies examining the influence of hypercapnia on acid-base balance, an increase of this magnitude causes a severe disruption to the normal acid-base balance of the haemolymph (e.g. *Carcinus maenas*, Truchot, 1975a; *Callinectes sapidus*, Cameron, 1978). It is possible that this factor could be partly responsible for some loss of condition and the eventual mortality of the animals in the system.

The very high levels of ammonia seen in the blood of the air-exposed animals were very quickly reduced in the sea water system. However, blood ammonia concentrations remained elevated at about

twice the values of control animals in fresh, running sea water. The ammonia concentration in the water of the recirculating system was, at times, very high (~0.3 $mmol.l^{-1}$) and liable to be detrimental to the viability of the animals. At the end of Run 1 and Run 2, the ammonia concentration, at around 0.06 - 0.07 $mmol.l^{-1}$, was more than 10 times higher than that normally present in fresh sea water.

The rapid rise in seawater ammonia concentration at the beginning of Run 2 must have resulted from a combination of factors:

1. Dumping from the blood and tissues of ammonia accumulated during the period of aerial exposure.
2. Its continual production by rock lobsters at an initially high rate due to their disturbed state and consequent high metabolic rate.
3. Bacterial decomposition of faecal material while the animals were clearing their gut contents.
4. Decomposition of animals which died before they were noticed or removed.

In any event, it is obvious that the rate of ammonia production initially far exceeded the capacity of the shell filter to remove it. The later drop in ammonia levels, even before most of the animals were removed, clearly indicates that the filter was functioning effectively at this time. This reversal may be partially due to a reduction in the input of ammonia into the water. It is also likely that the filter itself improved its operating performance. Just before the beginning of the second run the filter had been renewed and expanded in size to take the whole of the water circulation. Such shell filters rely on the establishment of a film of bacteria on the surface of the shells. It seems likely that during the period when the ammonia level was out of control the bacterial population was rapidly growing. The lag in acclimation time of biological filters has been recognised by Malone and Manthe (1985), who reported that between 25 - 40 days was required for biofilters in blue crab

shedding systems to control nitrogen (NH_3 and NO_2) levels. It is probably unwise to renew more than half of the shells on a single occasion so that an active population of bacteria is always present. Even so, it may be inevitable that there is a considerable lag in the response of the filter to a sudden increase in ammonia because bacterial growth probably responds to an increase in ammonia, and slows as ammonia is reduced.

Conclusions and Recommendations

The interpretations made are inevitably rather speculative. However, it is possible to make some general comments on the changes occurring in this particular system and possible improvements which could be made to its operation.

1. Salinity change was not significant and oxygen depletion was probably not a major problem either, although it could occur secondarily to another malfunction such as a rise in temperature or bacterial growth. Good aeration is also important to reduce the pH drop caused by carbon dioxide and may also help to eliminate ammonia, and therefore nitrite, as gas.
2. Any animal which dies should be removed as soon as possible. Crustaceans decompose very quickly, and could rapidly increase ammonia levels, bacterial contamination and oxygen depletion. Those in the lowest tiers are most likely to succumb. Thus the tanks should be arranged to allow convenient regular inspection and removal of moribund animals. From this point of view the closed recirculating system is inherently unstable. Once one or more of the animals die (from whatever cause) the whole system could rapidly deteriorate, which would be difficult to correct.
3. Once rock lobsters lose condition they do not readily recover, even in fresh, flowing sea water.
4. The drop in seawater pH was probably due to CO_2. Additional

aeration could help to raise seawater pH by driving off CO_2 as a gas. It was also suggested in the open forum of the National Symposium of the Blue Crab Fishery that NaOH or $NaHCO_3$ could be added to raise pH (moderator W.A. van Engel, 1985). Chemical additives should be treated with caution, since excess would result in the water becoming alkaline, which is probably as detrimental to the animals as water that is acidic.

5. Ammonia build up seems to be a problem at times and may be a major cause of the loss of condition. Further investigation of this factor is desirable and attempts made to improve its control. No more than half of the shell filter should be renewed at one time. Activated charcoal, which is often used to remove gases in biofilters, could be tried as an additional (but not alternative) measure.

6. Regular monitoring of ammonia, pH and oxygen in the sea water would be of benefit. A picture of the dynamics of the system would be assembled which might suggest ways of anticipating problems while they can still be corrected. Measurements should concentrate on the lowest tiers.

7. The most critical part of the whole cycle is during the first day when the animals are paying off an oxygen debt. At this time they are most active, most stressed, voiding faeces and some may die due to factors associated with capture and handling. It is almost certainly better to operate a flow through system, or at least change the water frequently, during this initial phase until the animals stabilise physiologically and the major risk of disastrous contamination of the system is over. The system could then be closed off to allow the temperature to be adjusted to that required for live shipment.

CHAPTER 7

SUMMARY AND CONCLUSIONS

The studies presented throughout this work have examined the effects of a variety of conditions on metabolism, ventilation, circulation and haemolymph acid-base regulation in the New Zealand rock lobster, *Jasus edwardsii*. The major points are summarised and discussed below, and potentially fruitful lines of research are indicated.

During progressive hypoxia *Jasus* regulated $\dot{M}O_2$ down to P_IO_2 = 80 Torr, below which it became dependent on the ambient oxygen tension. At levels below $P_{(crit)}$ f_{sc} and $\dot{V}_w$ still continued to increase, although at a slower rate than at higher oxygen tensions. In contrast to the pattern shown by many other crustaceans during hypoxic exposure, %Ext decreased progressively down to ~80 Torr, but below that level it remained constant at around 13% extraction of oxygen. This result suggests a limitation to efficient gas transfer during hypoxia.

Metabolic rate in resting submerged lobsters was comparatively low given the size of the animals and temperatures used. The increase in $\dot{M}O_2$ following exercise was not large, but there was little change in haemolymph [lactate]. It is suggested that high-intensity activity, in the form of tail-flips, is supported mainly by aerobic respiration.

Emersion (Chapter 4) and emersion following exercise (Chapter 5) both resulted in a reduced level of oxygen uptake, which in both cases was associated with an initial bradycardia. This contrasts with the changes in $\dot{M}O_2$ and f_H following exercise in water, where increases in both variables were observed (Chapter 3). Total

scaphognathite rate (f_{sc}) increased initially in all three experiments. However, while it subsequently decreased after exercise in both water and air, f_{sc} remained elevated during undisturbed emersion. These responses suggest that there are different factors controlling f_{sc}. The change occurring in air after exercise appears anomalous, and apparently relates to factors other than oxygen delivery. Possible modulating factors are discussed further in Chapter 5.

Exercise in water and air, and undisturbed emersion all induced significant mixed respiratory and metabolic acidoses. The source of the acid was qualitatively and quantitatively dissimilar in the three treatments. Following exercise in water the decrease in haemolymph pH resulted mainly from an increase in the concentration of metabolically produced hydrogen ions (H^+_m), while elevated levels of respiratory and metabolic H^+ equally contributed to the considerable pH depression following exercise in air. By contrast, there were apparently no metabolic acids released into the haemolymph during emersion, and indeed addition of base compensated partially for the effects of the respiratory acidosis.

These changes are related, at least in part, to variations in the concentration of haemolymph bicarbonate. There was essentially no change in the concentration during air exercise, but it fell after exercise in water and increased during emersion. Thus the ability to counteract acids was different in the 3 treatments. Comparison of the changes in the metabolic acid load (ΔH^+_m) with the lactate load (ΔLa^-) showed quantitative and qualitative differences in these variables in the three treatments. Following exercise and recovery in water, ΔH^+_m was at all times greater than ΔLa^-, it was much less than ΔLa^- during emersion, and the two were approximately equal, at least over the first 4h, during air exposure post-exercise (Figs. 3.12, 4.9 and 5.11). The data suggest different release dynamics and buffering in

the three treatments. *Jasus* has the apparent ability to retard either hydrogen ion or lactate ion release from the tissues.

It is suggested that the ultimate factor governing each of the responses is the effective functioning of the gills in the two media. Ion and gas exchange are severely limited during air exposure, but are able to function when the animals are in water.

In all of the experiments conducted here, *Jasus* showed a very low resting concentration of bicarbonate (approximately 3.5 meq.l^{-1}), which was only about one-third of [HCO_3^-] in *Homarus* (Taylor and Whitely, 1989). The ability to regulate haemolymph pH appears to be due to the low, maximum bicarbonate concentration.

There was a considerable variability in the amount of oxygen used post-treatment in the different experiments. The O_2 debt was lowest after hypoxia (1.4 mmol $O_2.kg^{-1}$) and highest during recovery from exercise in air (15.6 mmol $O_2.kg^{-1}$). The amount of oxygen used during recovery in any of the experiments was not directly comparable with variations in either haemolymph lactate or $\dot{M}O_2$ during treatment. In all three treatments, the calculated lactate portion (based on the concentration in the haemolymph) of the oxygen debt was low at 5-20% of the total debt, suggesting a large alactic part. More reliable estimates of the metabolic cost of anaerobic respiration could be achieved through measurements of the whole body lactate concentration.

Jasus seems highly sensitive to changes in either environmental or internal conditions. This was most graphically illustrated in animals held prior to export in recirculating sea water (Chapter 6), where the animals became moribund, and some died. Variations in seawater quality, particularly the low pH and high ammonia concentration, were probably responsible for the high mortality. Transferring the animals to fresh sea water did not alleviate the problem, indicating that the changes initiated when the animals were

in the recirculating system were not reversible.

A number of questions arise from this study and provide excellent topics for future research. Particularly noteworthy are the changes occurring in lactate - is it retained intracellularly or removed to another compartment? Is there in fact another metabolite produced during anaerobic metabolism in this species? The changes observed in scaphognathite and heart rate also imply that several mechanisms control their function. What mechanisms are involved in changing cardiorespiratory frequencies? The reduction in %Ext during hypoxia was also surprising - what is the factor limiting conductance during exposure to low oxygen tensions? Because of difficulties associated with clotting, haemolymph oxygen levels were not measured simultaneously with other variables in this study. However, it is likely that technical difficulties could be overcome in a separate experimental series. Such measurement might lend weight to, or refute, a number of speculative interpretations advanced in this thesis, and would, no doubt, stimulate further questions regarding respiration and acid-base regulation in this species.

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